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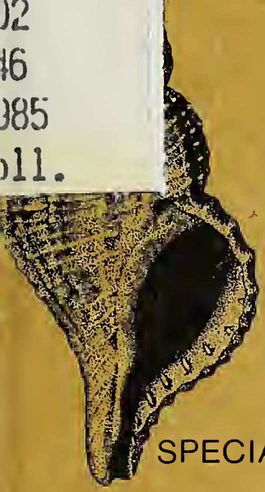

PERSPECTIVES IN MALACOLOGY

SPECIAL EDITION NO. 1 OF THE *AMERICAN MALACOLOGICAL BULLETIN*
HONORING PROFESSOR MELBOURNE R. CARRIKER

SPECIAL EDITION NO. 1 JULY 1985

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Cover. *Urosalpinx cinerea* (Say), the Atlantic oyster drill, drawn by Melbourne R. Carriker.

THE AMERICAN MALACOLOGICAL BULLETIN (formerly the Bulletin of the American Malacological Union) is the official journal publication of the American Malacological Union.

AMER. MALAC. BULL. SPECIAL EDITION NO. 1
July 1985

EDITORIAL COMMENT

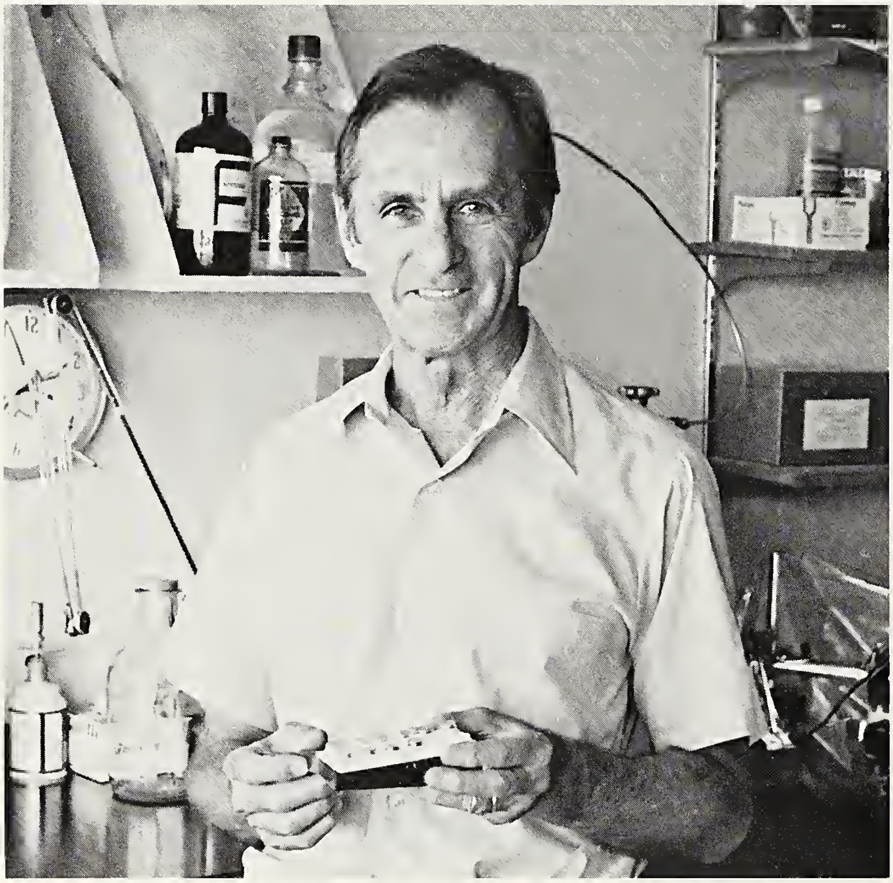
With the publication of *Perspectives in Malacology*, the *American Malacological Bulletin* embarks upon another new phase of its development. Through the publication of special editions, such as this and the forthcoming *Proceedings of the Second International Corbicula Symposium*, and *Entrainment of Larval Oysters*, we hope the *American Malacological Bulletin* will continue to serve as an outlet for timely and important communications of advances in Malacology.

These Special Editions will highlight recent malacological events and symposia. This first edition honors Professor M. R. Carriker on the event of his nominal retirement. Each participant of this Symposium Proceedings has in one way or another been influenced by Professor Carriker as co-worker, student, friend, or with luck, all three. The Editors of this Special Publication have been among the lucky ones and are thus particularly honored to have this opportunity to pay homage to their mentor.

We also take this opportunity to acknowledge Dr. L. Leon Campbell, Provost of the University of Delaware, for his continued support of this endeavor. The University of Delaware has kindly financed this publication because of Dr. Campbell's support. Dr. Franklin C. Daiber also deserves special thanks for his adept organization and coordination of this Symposium.

We are pleased to have the chance to start a new series of special publications in honor of Professor Carriker and hope this and future special editions will prove to be strong additions to our growing stable of AMU publications.

Editors
Perspectives in Malacology



PROFESSOR MELBOURNE R. CARRIKER

PERSPECTIVES IN MALACOLOGY; A SYMPOSIUM TO HONOR M. R. CARRIKER

FOREWORD

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This special issue of the *American Malacological Bulletin* is dedicated to Melbourne Romaine Carriker. The papers that make up this issue result from a symposium held on Thursday, February 7, 1985 on the campus of the University of Delaware to honor the time of his retirement and to give recognition to his many acccomplishments.

Mel, as he is fondly called by friends and associates, was born 25 February 1915 at Santa Marta, Colombia, South America, where his family owned a coffee plantation. His introduction to zoological studies came at an early age. During 1934, he was a member of a nine month ornithological expedition to the eastern Andean slopes of Bolivia sponsored by the Academy of Natural Sciences of Philadelphia and led by his father, Melbourne A. Carriker, Jr.

Dr. Carriker received his Bachelor of Science degree at Rutgers University in 1939 and his Ph.D. in 1943 in invertebrate zoology and physiological chemistry from the University of Wisconsin. He was granted an honorary Doctor of Science degree from Beloit College, Wisconsin in 1968.

His scientific career was interrupted during the war years when he served as a U.S. Naval Reserve officer in the North Pacific (1943-1945). Following the war he became Assistant Professor of Zoology at Rutgers University where he rapidly established himself as a master of molluscan comparative anatomy and functional morphology. He began long term investigations of shell-boring mechanisms of predatory gastropods in general, but of *Urosalpinx* in particular, the published results of which have been highly acclaimed. Mel served as Associate Professor of Zoology at the University of North Carolina from 1954 to 1961 and during 1961-62 he was Program Director for the U.S. Bureau of Commercial Fisheries at the Biological Laboratory, Oxford, Maryland. Subsequently he spent a decade directing the ambitious and highly valued Systematics-Ecology Program at the Marine Biological Laboratory at Woods Hole, a program that resulted in numerous publications through which our knowledge of the marine flora and fauna of the Northeastern United States has been greatly increased. Mel has continually served from 1965 as editor for the manuals produced by the National Marine Fisheries Service on the Marine Flora and Fauna series. In 1973, he was appointed Professor of Marine Studies at the College of Marine Studies, University of Delaware, based at the Lewes Marine Studies Complex. It was here that Dr. Carriker reached full stride as a marine biologist, malacologist, teacher, editor, nationally and internationally involved leader.

Mel's career can be characterized as one of continual growth, diverse and multifaceted. It can be viewed through the eyes and with the minds of his students, and colleagues with whom he has interacted. Hallmarks of his career have been his dedication to students, mentor to associates, enthusiasm for science, outstanding service to his institutions and community, dedication to scientific excellence, organizer, editor, communicator, and international scientific ambassador of good will.

While he has officially retired, it is in name only. He continues to participate and to serve as he has for more than forty years.

FOUR CHARACTERS AND THE HIGHER CATEGORY SYSTEMATICS OF GASTROPODS

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ABSTRACT

Four characters are shown to have fairly similar patterns of taxonomic occurrence in a diverse array of mainly marine Gastropoda. These patterns and the morphological and presumably functional uniformity support the ideas that, within themselves, the characters are homologous and reflect phylogeny. The characters are: 1, *pigmented mantle organs* (PMOs) in the dorsal or right lateral mantle cavity wall of larvae and (in some taxa) postlarvae; 2, longitudinal *ciliated strips* (CSs) dorsal and ventral in the mantle cavity of postlarvae, merging posteriorly (commonly replacing ctenidia or other "gills" in creating the flow of water through the mantle cavity); 3, *chalazae* (CHae) connecting egg cocoons one to another in a gelatinous egg mass or capsule, and 4, hyperstrophically coiled larval shells *(heterostrophy* [HET]).

In veligers, PMOs have almost invariably been thought to be excretory (larval kidneys). They are retained through metamorphosis into adulthood in the Epitoniacea, Architectonicidae, and Pyramidellidae. Also, they are retained sporadically in some young postlarval Bullomorpha. In postlarvae, PMOs have often been called hypobranchial glands. PMOs have not been shown to be excretory or hypobranchial in structure or function, and it seems highly unlikely that they change either of these at metamorphosis. PMOs release fine particles in suspension when the animal metamorphoses or is stressed. A PMO is thus unlike the purple-secreting true hypobranchial gland of muricaceans, the prey-anesthetizing dye of which is released in solution and becomes purple only after exposure to oxygen and light. The colors of the PMO pigments, which range from black, purple and brown to yellow, are in some cases genus- or species-specific. A histological cross-section of the mantle cavity, PMO, and two CSs of postlarval *Fargoa bartschi*, a pyramidellid, is illustrated as a basis for future comparisons.

Data on the taxonomic occurrences of the four characters (with larval and postlarval PMOs distinguished) are tabulated and summarized. The characters occur doubtfully in a few "primitive" mesogastropods. They occur definitely in some or all Epitoniacea, Architectonicacea, Pyramidellidae, Bullomorpha, Thecosomata, Aplysiomorpha, Pleurobranchomorpha, Ascoglossa, and Basommatophora, *i.e.* taxa in all three subclasses of the Gastropoda. It is well-established that "primitive" Opisthobranchia and "primitive" Pulmonata (Euthyneura) are related, being similar to one another anatomically and functionally. Hitherto, it was believed that the Euthyneura were derived from archaeogastropods or "primitive" mesogastropods (cerithiaceans, rissoaceans, or littorinaceans). This paper shows that the ancestor or ancestors of euthyneurans probably also had some characters retained in living prosobranchs primarily in the Epitoniacea, Architectonicacea, and Pyramidellacea — which are now (and always have been?) far too specialized to be ancestral to gastropod higher taxa, which should be characterized by *combinations* of many more than four characters.

Over twenty years ago I began intermittent study of the natural history of the Epitoniidae, Architectonicidae, and Pyramidellidae. Aspects of particular interest to me are their feeding, reproductive biology, larvae, and systematics (including higher categories). The three families are of phylogenetic interest because they combine prosobranch and opisthobranch traits (Robertson, 1974). This paper presents and reviews certain data from the egg masses, veligers, larval shells, and postlarval anatomies of the three families and what I believe to be their closest living relatives (in different superfamilies, orders, and even subclasses). Four characters are shown to be relevant to an overall understanding of gastropod higher category relationships, and of the evolutionary origins of the extraordinary diversity in gastropod form and function.

American Malacological Bulletin, Special Edition No. 1(1985):1-22

Studying the veligers of all three families, I noted the consistent presence of a conspicuous pigmented organ. This is variously colored (commonly black, brown, purple, or yellow) or two- or even three-colored, and is located in the dorsal or right lateral mantle cavity wall under the shell near the anus. In the literature, these organs have been called larval excretory organs (kidneys). Having doubts that this is their function, since 1973 I have noncommittally been calling them *pigmented mantle organs* (PMOs).

My own (partly unpublished) and several other studies have shown that in epitoniids, architectonicids, and pyramidellids, PMOs in the veligers are retained with little or no change through metamorphosis and throughout postlarval life, maintaining approximately the same relative size. In the literature, the PMOs of adults have been called, among other things, hypobranchial glands. A larval kidney can hardly transform at metamorphosis into a hypobranchial gland!

The PMO has not been shown to have the histology or functions of an excretory organ or a hypobranchial gland. The idea that the PMO is hypobranchial in postlarval epitoniids, architectonicids, and pyramidellids derives from its release of pigment that in some cases is purple. At least two more crucial differences between hypobranchial and PMO colors have been overlooked. In muricids, for example, the true hypobranchial gland secretes an indigo-like dye in solution that is first greenish yellow and then becomes purple upon exposure to oxygen and light (Fretter and Graham, 1962). The PMO, by contrast, consists of a thin-walled, easily-ruptured sac full of paste-like material that does not change color (except rarely during ontogeny). This pigment is released as a cloud of fine particles in suspension. These particles have yet to be characterized chemically (but see Comfort, 1961). The second difference is that the purple of muricaceans is used to anesthetize prey. In the Bahamas, I have observed *Purpura patula* (Linné) using its purple to attack the chiton *Acanthopleura granulata* (Gmelin). By contrast, repeated observations have shown that the PMO of postlarval epitoniids does not release pigment before or while the animal feeds (*e.g.*, Robertson, 1983a,b). Pigment is released when the animal metamorphoses, or is molested, nearly drying out, dying, or dropped into alcohol. Further discussion of a possible repugnatorial role of the PMO is beyond the scope of this paper.

(Parenthetically, it should be mentioned that there is a third major kind of purple dye released by gastropods, aplysiopurpurin, coming from postlarval *Aplysia*. This is released in solution and does not change color. Its chemistry has been shown to differ radically from that of muricid purple. Judging by Comfort (1961), aplysiopurpurin is not the same as a PMO pigment (ianthinine, from *Janthina*). *Aplysia* does, however, release its dye when it is stressed.)

Thorson (1946) was struck by the similarity between the PMOs of larval pyramidellids and tectibranchs. These and other data led to Fretter and Graham's (1949) transfer of the Pyramidellidae from the Prosobranchia to the Opisthobranchia. Thus PMOs have already been used to infer phylogenetic relationships. Thorson later (1957) noted that larval epitoniids also have pyramidellid- and tectibranch-like PMOs.

In the mantle cavities of adult architectonicids and pyramidellids, there are paired and juxtaposed longitudinal *ciliated strips* (CSs) that join posteriorly. When ctenidia or other "gills" are reduced in size or absent, CSs create the left to right flow of water through the mantle cavity. CSs are beside and below the (shorter) PMO, and they help to stir the particles into suspension when the PMO is ruptured. CSs seem not to be present in any veligers or epitoniids of any age.

Similar (homologous?) PMOs and CSs also occur in a wide array of mainly opisthobranch gastropods, the PMOs primarily in veligers and the CSs in postlarvae. CSs also occur in certain postlarval basommatophorans. I became intrigued by the possibility that the pattern of taxonomic occurrence of each of these characters might yield evidence on whether there are homologies from taxonomic group to group. This would support the idea that these characters are relevant to an understanding of higher category relationships. I therefore assembled the data in Tables 1-3 from my own work and the literature.

Two characters are obviously too small a sample to achieve the desired ends, so two more characters already familiar to me were added to this study. These are *chalazae* (CHae), connecting egg cocoons end to end within an egg mass or capsule (Table 4), and *heterostrophy* (HET), larval hyperstrophy (Table 5).

Explanation is needed as to why pyramidellids are here classified as prosobranchs. After transferring the family to the opisthobranchs in 1949, Fretter and Graham treated it extensively in their classic 1962 book on British *prosobranchs*, tabulating some of the prosobranch, transitional, and opisthobranch traits of pyramidellids on p. 639. Grouping pyramidellids as opisthobranchs separates them from their closest living (prosobranch?) relatives, *i.e.* architectonicids and more distantly epitoniids. Morton (1963:56) wrote: "In their totality of characters, the Pyramidellidae are somewhat unlike any classical opisthobranch." Fretter (1980) stated that no family but pyramidellids "has evolved a mosaic of opisthobranch-prosobranch characters ...," and that this family is the only one to have "turned ... the balance towards opisthobranchs ..." Fretter (1980) divided the Opisthobranchia into the Pyramidellimorpha and Euopisthobranchomorpha, thereby implicitly acknowledging differences. Most recently, Gosliner (1981), a student primarily of opisthobranchs, gave reasons for excluding pyramidellids from the subclass. I concur, but the question is not so much "Which subclass does this family belong in?" as "Where should the line be drawn between the two intergrading subclasses?"

Explanation is also needed as to why the order (or suborder) Heterogastropoda does not appear in the tables. The taxon was named and defined in an illustrated popular shell book in Japanese by Habe and Kosuge

(1966) and further discussed in a scientific paper in English by Kosuge (1966). Recently, some other malacologists (*e.g.* Climo, 1975; Fretter and Graham, 1982) have, formally or informally, been recognizing the Heterogastropoda. I quote from Robertson (1974:215):

> [Habe and Kosuge] grouped the Architectonicidae, Mathildidae, Epitoniidae, Janthinidae and Triphoridae in ... the Heterogastropoda, which they placed between the Neogastropoda and Basommatophora; they dispensed with subclasses and placed the Entomotaeniata (Pyramidellidae) and Cephalaspidea [Bullomorpha] after the Basommatophora. This classification is unsatisfactory because the relationships between Triphoridae and the other 4 families seem highly tenuous and because the other families are separated from their nearest relatives (mesogastropods, pyramidellids and cephalaspids).

In 1980, Fretter did not consider the heterogastropods "to be a valid group of gastropods, but a convenient descriptive term for those which may have followed some initial steps towards the opisthobranch grade." Fretter and Graham (1982) extended the concept of the 'Heterogastropoda' to include the Cerithiopsacea and Eulimacea, but they persisted in excluding the Pyramidellidae. Boss (1982) did include the Pyramidellidae in the Heterogastropoda provisionally, but he excluded the Cerithiopsidae, Epitoniacea, Aclididae and Eulimacea (all six taxa were grouped as prosobranchs).

In a paper mainly on one character (osphradial fine morphology), the Architectonicidae and Pyramidellidae were grouped by Haszprunar (1985b) in a new higher taxon (order?) Allogastropoda, which he placed in the subclass (?) Heterobranchia (=Euthyneura). He did not characterize these or (Haszprunar, 1985a) the suborder Heteroglossa (his new name for the Heterogastropoda), but referred for supporting evidence to two of his papers still in press. As these are not yet available to me, I pass judgment on the need for these higher taxa. It seems improbable, though, that a good case can be made for the Architectonicidae and Pyramidellidae being "the most primitive members of recent Heterobranchia [=Euthyneura]" (Haszprunar, 1985b:504).

Organismic nomenclature in the tables in many cases is updated. Taxa are identified only down to genera except in the case of previously unpublished data, for which species names are given. The classification and nomenclature of prosobranchs mainly follow Taylor and Sohl (1962), of opisthobranchs Thompson (1976), and of basommatophorans Hubendick (1978). Tillier's (1984) reclassification of the lower pulmonates arrived too late

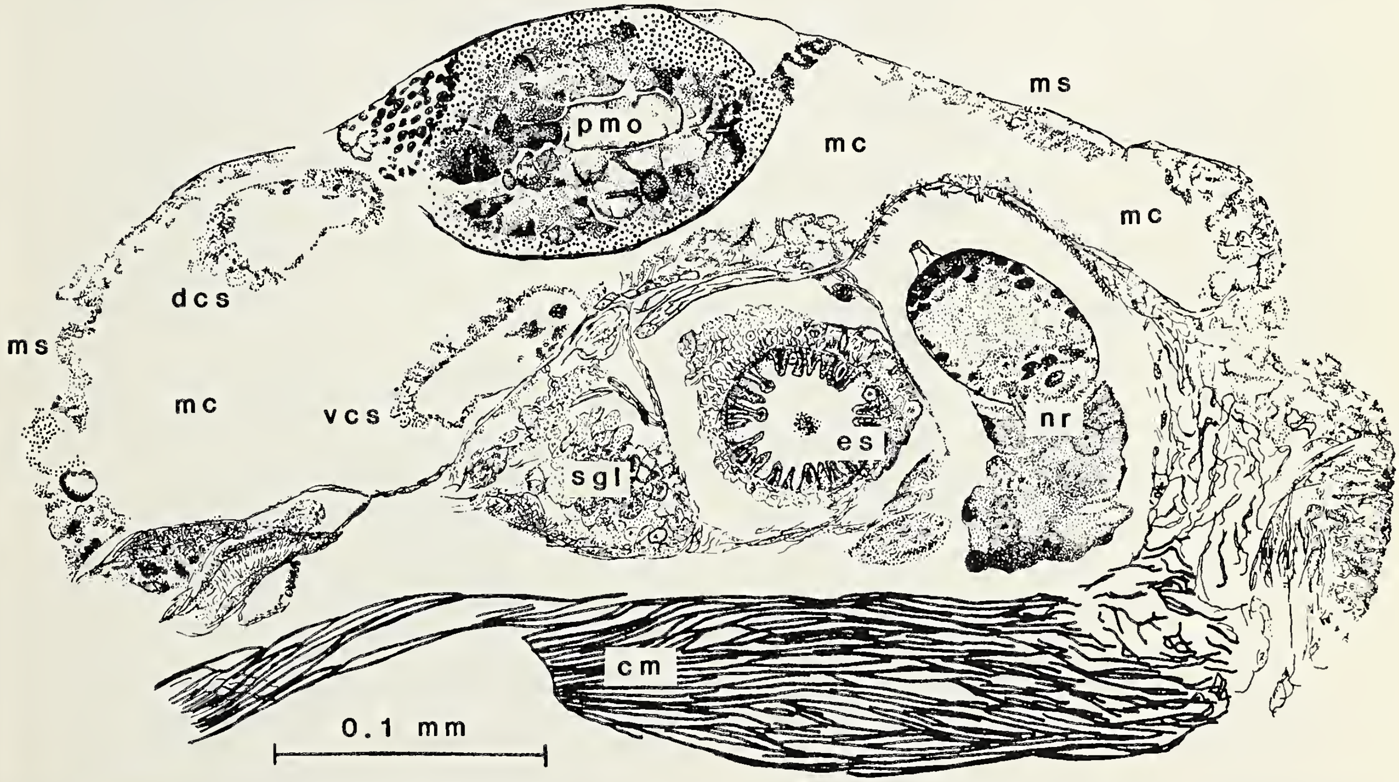

Fig. 1. Histological cross section through the mantle cavity (mc) of an adult odostomioid pyramidellid, *Fargoa bartschi*, showing the pigmented mantle organ (pmo) and the nearby dorsal and ventral ciliated strips (dcs, vcs), which are shown to be hollow crests that are attached weakly to the mantle cavity walls. The animal's left and right are transposed. In this species the periphery of the PMO is yellow and the interior purple. Other abbreviations: cm, columellar muscle; es, esophagus; ms, mantle skirt; nr, nerve ring; sgl, salivary gland.

Table 1. Systematic list of gastropods hypothesized to have homologous pigmented mantle organs (PMOs) either in veligers or postlarvae.

Taxon	Observed in veliger (V) or postlarva (PL)	Term applied	Color	Reference
PROSOBRANCHIA				
Mesogastropoda				
Superfamily?				
Rissoellidae?	PL	blotches	dark	[Note 1]
Epitoniacea ("Ptenoglossa")				
Epitoniidae	PL	Manteldrüse	purple-like	Thiele, 1928
Epitoniidae	PL	hypobranchial gland	purple	Robertson, 1963
Epitoniidae	PL	pigmented mantle organ	?	Robertson, 1974
Epitoniidae	V	pigmented mantle ("excretory") organ	?	Robertson, 1976
Couthouyella [Note 2]	PL	hypobranchial gland	purplish	Warén, 1980a
Epitonium	V	excretory organ	bluish-purple	Thorson, 1946 [in part as *Aclis*; Note 3]; Thorson, 1957
Epitonium, *Amaea*, etc.	PL	purple gland	ashy brown	Taki, 1956, 1957
Epitonium	PL	hypobranchial gland	purple	Fretter and Graham, 1962
Epitonium	PL	hypobranchial gland area	purple	Salo, 1977
Epitonium	V & PL	purple gland (V); hypobranchial gland (PL)	dark purple	McDermott, 1981
Epitonium	PL	hypobranchial gland	purple	Fretter and Graham, 1982
Janthinidae	V	pigmented mantle ("excretory" organ)	?	Robertson, 1976
Janthina	V	pigment mass	violet	Haddon, 1882
Janthina	PL	Hypobranchialdrüse; Manteldrüse; Farbdrüse	purple-like	Thiele, 1897
Janthina	V	Farbdrüse	?	Fraenkel, 1927
Janthina	PL	hypobranchial gland	purple; violet	Fretter and Graham, 1962, 1982
Architectonicacea				
Architectonicidae	V	pigmented mantle ("excretory") organ	?	Robertson, 1976
Heliacus spp.	V & PL	PMO	black; pink; yellow; violet	Robertson, unpublished
Philippia	V & PL	(opisthobranch-like) larval excretory organ	black	Robertson *et al.*, 1970
Mathildidae?				
Gegania?	PL	sac-like organ [Note 4]	dark	Climo, 1975
Pyramidellacea				
Pyramidellidae (including Cyclostremellidae [Note 5])		pigmented hypobranchial gland	?	Fretter and Graham, 1962
Pyramidellidae	V & PL	PMO	usually dark	Robertson, 1974
Pyramidellidae	V	pigmented mantle ("excretory") organ	?	Robertson, 1976
Boonea 3 spp.	V & PL	PMO	yellow and brown	Robertson, unpublished
Cyclostremella	PL	PMO (?hypobranchial gland)	greenish white, bright yellow, and brown	Robertson, 1973
Ebala	V	larval kidney	reddish-yellowish	Rasmussen, 1944
Ebala	V	excretory organ	sealing-wax red; brownish; dark reddish purple	Thorson, 1946
Fargoa bartschi	PL	PMO	yellow-brown (juv.); dark brick red (adults)	Robertson, 1978
Fargoa 3 spp.	PL	PMO	yellow and brown	Robertson, unpublished
Odostomia	V	glande anale	?	Pelseneer, 1914
Odostomia	V	anal gland	nearly black	Lebour, 1932
Odostomia	V	larval kidney	grey	Rasmussen, 1944
Odostomia	V	excretory organ		Thorson, 1946
Odostomia	V & PL	pigmented gland	brown-orange or pink	Fretter and Graham, 1949
"*Odostomia*"	V	excretory organ	black	Amio, 1959
Odostomia	PL	Excretionsorgan	orange-red	Ankel, 1959

Table 1. (continued)

Taxon	Observed in veliger (V) or postlarva (PL)	Term applied	Color	Reference
Odostomia	PL	Larvenniere	orange-red	Maas, 1965
"*Odostomia*"	V	[larval kidney]	dark	Minichev, 1971
Pseudoskenella	PL	[colored] region of hypobranchial gland	yellowish orange	Ponder, 1974
Pyramidella crenulata		PMO	pale yellow	Robertson, unpublished
Turbonilla	PL	protein cells of hypobranchial gland	?	Fretter and Graham, 1962
Turbonilla sp.	PL	PMO	pale greenish yellow and pale brown	Robertson, unpublished
Turbonilla vineae	PL	PMO	pale greenish yellow	Robertson, unpublished
OPISTHOBRANCHIA ("Euthyneura" in part)				
Bullomorpha ("Cephalaspidea")				
Many families	V	excretory organ (kidney)	orange-red; black; carmine (V); purple; red	various, e.g. Thorson, 1946; Hurst, 1967
Diaphanidae	PL	pigmented hypobranchial gland	?	Fretter and Graham, 1962
Retusidae				
Retusa	V & PL	larval (secondary) kidney	black	Smith, 1967
Aglajidae				
Aglaja	V	[PMO]	brown-black	Fasulo *et al.*, 1982
Philinidae				
Philine	V	spot (excretory organ)	red, later black	Amio, 1963
Philine	V & PL	larval kidney	jet black	Horikoshi, 1967
Philinoglossidae				
Philinoglossa [Note 6]	V	larval kidney	black	Swedmark, 1968
Pleurobranchomorpha ("Notaspidea")				
Umbraculidae				
Umbraculum	V	Exkretionsorgan	brown	Heymons, 1893
Umbraculum	V	large . . . spot	brown	Ostergaard, 1950
Pleurobranchidae				
Berthella	V	anal cells	dark	Usuki, 1969
Berthellina	V	larval kidney	dark reddish	Gohar and Abul Ela, 1957
Pleurobranchus	V	excretory organ	pink	Rao and Alagarswami, 1962
Ascoglossa ("Sacoglossa")				
Several families	V	excretory organ (kidney)	black	various, e.g. Thorson, 1946; Hurst, 1967
PULMONATA? ("Euthyneura" in part)				
Basommatophora?				
Amphibolidae?				
Amphibola?	V	larval kidney	black	Pilkington and Pilkington, 1982 [Note 7]

for consideration here.

MATERIALS AND METHODS

The main places where epitoniids, janthinids, architectonicids, and pyramidellids were studied alive are listed in the Acknowledgments. The species are listed in the tables.

Several North American east and Gulf coast odostomioid pyramidellids were studied histologically. The most instructive section was from *Fargoa bartschi* (Winkley, 1909), a "rare" ectoparasite on the serpulid polychaete *Hydroides dianthus* (Verrill, 1873). For information on the systematics, spermatophores, and feeding of *Fargoa* and *Boonea*, see Robertson (1978) and Robertson and Mau-Lastovicka (1979). The specimen of *F. bartschi* came from less than 1 m below low tide mark on the shore adjacent to Flume Pond, S of Gunning Point, 4 km NNE of Woods Hole, and was collected on July 13, 1978. The animal was relaxed with a solution of $MgCl_2$ isotonic with sea water, and fixed in Ciaccio (as suggested by Dr. Vera Fretter). Sections were cut 7 μm thick, and stained with hematoxylin and eosin.

RESULTS

PIGMENTED MANTLE ORGANS (PMOs)

These are here defined as nearly spherical to elongate, thin-walled, vacuole-containing sacs, each in a dorsal or right lateral mantle cavity wall, near the anus and under the shell (often visible in transparency). The sac contains paste-like pigment particles (on their colors see later). When the sac ruptures, particles are released in a cloudy suspension. Care has to be taken not to identify as PMOs pigment markings having other attributes and positions.

Figure 1 illustrates a histological cross section of the mantle cavity (mc) of the odostomioid pyramidellid *Fargoa bartschi*, showing its PMO and nearby CSs (see later). The section is anterior to the anus, at the level of the nerve ring (nr) and salivary gland (sg). Fretter and Graham's (1962: fig. 79, pc) cross section of the mantle cavity of a *Turbonilla* (Pyramidellidae) is one of the few published histological portrayals of a PMO. Their figure shows more discrete cell outlines, and they identify them as "protein cell[s] of [the] hypobranchial gland." Farther to the animal's right, above the dorsal CS, they show "mucous cell[s] of the hypobranchial gland." Fretter and Graham also show something else not seen in *Fargoa*: "hypertrophied gland cells on [the] floor of [the] mantle cavity."

The "purple gland" (PMO) in postlarval Epitoniidae is long and cylindrical and lies to the right of the ctenidium and to the left of the oviduct ("vagina") and (posteriorly) the rectum ("intestine") (Taki, 1956, 1957). The purple-secreting "hypobranchial gland" of *Janthina janthina* (Linné) is a broad band in the region between the "vas efferens" and the rectum; that of *J. exigua* Lamarck lies above the anterior end of the "uterus," which is anterior to the rectum (Thiele, 1897:647-648). In adult *Heliacus* (Architectonicidae), the PMO is fragmented by transverse folds of the mantle (a "gill"?) and extends between the dorsal CS and rectum (Robertson, unpublished). Generally, though, a PMO remains cohesive.

PMOs occur in many veligers of taxa in the mesogastropod - lower euthyneuran grade (Table 1). Histologically, the PMO of a larval *Retusa* (Bullomorpha) is like that of postlarval *Janthina*, *Fargoa*, and *Turbonilla* (Smith, 1967). In larval *Philine* (also Bullomorpha), however, the pigment appears to surround the PMO cells (Brown, 1934), but perhaps these had ruptured.

PMOs are retained through metamorphosis and throughout life in four or five prosobranch families: the Epitoniidae, Janthinidae, Mathildidae, Architectonicidae, and Pyramidellidae. Unfortunately, the relevant observations on metamorphoses showing that this is definitely the case remain largely unpublished (I am the principal culprit). Dr. Jane B. Taylor (unpublished) has made such observations on *Epitonium* (Epitoniidae) and *Janthina* (Janthinidae), and I have on *Heliacus* (unpublished) and *Philippia* (both Architectonicidae) (Robertson *et al.*, 1970); I have also on *Boonea* (Pyramidellidae) (unpublished). McDermott (1981:70) remarked that the "dark purple mass" in an *Epitonium* veliger "is probably the rudiment of the pigmented hypobranchial gland of the adult."

PMOs also occur in the young postlarvae (rarely adults) of at least two opisthobranch families, the Retusidae and Philinidae (Table 1). In the prosobranchs in which it occurs, the PMO maintains approximately the same size relative to the shell, although it often elongates after metamorphosis. In the few opisthobranchs that retain the PMO into the young postlarva, the PMO decreases in relative size and in most species disappears (see Thorson, 1946). Thus the prosobranch PMO condition seems more "primitive" than the opisthobranch one.

In *Philine*, a remnant of the "larval kidney" is visible in the postlarval juveniles of at least four species, and it is retained in the adults of one or more of these (Horikoshi, 1967). In *Retusa*, "the embryonic kidney degenerates ... after hatching" (Smith, 1967:751).

PMO pigments occur in nearly all colors in the spectrum: black, purple, violet, blue, greenish, yellow, grey, brown, orange, pink, and red. Commonly, one PMO is two- or even three-colored, one color partly masking another. The colors are usually genus- or species-specific (Table 1) so they are, in many cases, good taxonomic characters at the levels of species and genera. Only in *Philine* has a PMO color been reported to change during ontogeny. Combining all the available color information about the Pyramidellidae and all the other PMO-bearing taxa combined, it can be seen (Table 2) that there is about the same color diversity within this family as in all the others combined. Thus there is no basis for a claim that the color diversity reflects non-homology. There are taxonomic trends: yellow is commonest in the Pyramidellidae, purple in the Epitoniacea, black in the Architectonicacea and Bullomorpha, etc. *Janthina* is the PMO-bearing gastropod yielding the most copious quantities of PMO pigment. This happens to be purple or violet. Comfort (1961) did well to choose this animal for his preliminary biochemical study.

Table 2. PMO colors (from Table 1): approximate numbers of occurrences in different species or genera.

	Occurrences	
	Pyramidellidae	Non-Pyramidellidae
Yellow/orange	13	2
Red/pink	7	7
Brown	7	4
Black/grey	3	10
Greenish white	3	0
Purple/violet/blue	1	14

"Larval or secondary kidneys" are well known in aplysiomorph, nudibranch, and onchidiid veligers. These organs are like PMOs in being near the anus, but they mostly are colorless; occasionally, though, they have some pigmented inclusions. Heymons (1893), Mazzarelli (1904), Saunders and Poole (1910), Smith (1967), Thompson (1976:81), and Bonar (1978), among others, believed that these "larval kidneys" are homologous with PMOs, but this seems questionable. Fioroni (1966) provisionally distinguished between primary larval kidneys, cephalic larval kidneys, anal or pallial larval kidneys, and other larval excretory organs. PMOs belong in the third category, but Fioroni also included in it some possibly extraneous transparent or even paired structures. The best recent papers on the confused subject of opisthobranch "larval kidneys" are by Bonar and Hadfield (1974), and Bickell and Chia (1979). Hurst (1967) was the first to emphasize the absence of pigment in the "kidneys" of most larval "higher" opisthobranchs.

CILIATED STRIPS (CSs)

These are here defined as paired dorsal and ventral longitudinal tracts of strongly beating cilia extending from near the mouth of the mantle cavity to its posterior, near or at which the strips join. Figure 1 shows that, along part of their lengths, *Fargoa* CSs are close to the PMO. (The PMO is differently positioned in some other taxa.) The figure also shows that lumina underly some CSs. In *Heliacus*, the ventral CS lumen is the pedal gland that passes through the nerve ring and secretes the tough, elastic mucous thread emerging from the ventral pedal pore (Dr. George M. Davis, unpublished). In other taxa, the lumina may be blood sinuses. These strips and their posterior termination are present even in the extraordinarily long pallial cecum of *Acteon*, which coils to the tip of the viscera (Fretter and Graham, 1954). CSs commonly aid or replace ctenidia or "gills" in creating the left to right flow of water through the mantle cavity. In taxa with postlarval PMOs, CSs help to stir the PMO pigment particles into suspension. It is noteworthy that the taxon with the most copious PMO pigment (Epitoniacea) lacks CSs that would help to disperse it.

Of the four characters treated in this paper, CSs are the one most likely not to be homologous in all the animals in which they are here recorded (Table 3). Molluscan skin is commonly ciliated, and ciliary tracts might evolve convergently in places where there is a functional "need" for them. The "ciliated strips" of the minute prosobranchs *Omalogyra* and *Rissoella* may well be convergent with the other CSs in Table 3 (Note 8).

CHALAZAE (CHae)

The chalazae in a hen's egg are different from gastropod chalazae, the latter being a misnomer well entrenched in the literature. Gastropod chalazae are here defined as threads connecting egg cocoons (Thorson's 1946 term) end to end in a continuous string in the order in which they are laid, inside a gelatinous egg mass or capsule. There are one or more egg cells or embryos in a cocoon (they are most numerous in *Aplysia*). CHae help maintain the integrity of an egg mass, and they may also tether each cocoon to facilitate hatching of its contained larva(e).

A chalaza is continuous with the wall of the cocoon. According to Pelseneer (1914: fig. 5), chalazae of a European *Odostomia* (Pyramidellidae) have inner and outer layers, the inner one terminating at the *inner* end of the cocoon, interrupting the inner layer of the cocoon (shown black in Pelseneer's figure). The outer layer of the chalaza is continuous with the outer layer of the cocoon. Morton (1955c) made similar observations on the egg cocoons and CHae of *Ovatella* (Ellobiidae), except that he did not distinguish two layers in the cocoon walls, only fine concentric laminae. CHae are commonly thin and overlooked.

CHae occur almost exclusively in taxa of the "higher" mesogastropod - lower euthyneuran grade (Table 4). However, they have also been reported in *Valvata* (Valvatidae), an enigmatic group generally classified somewhere among "lower" mesogastropods, and *Campanile* (Campanilidae), a cerithiacean also in the "lower" mesogastropods.

The best published work on *Valvata* "chalazae" is by Kruglov and Frolenkova (1981), who did not realize their phylogenetic interest. *Valvata* lays its cocoons in egg capsules, which by my definition have hardened flexible outer walls. All other CHae recorded in Table 4 are from gelatinous egg masses. A capsule necessitates the presence of a preformed exit hole or slit through which the young can hatch. A slit is present on a *Valvata* capsule. CHae connecting the cocoons are attached inside the base and apex of the capsule. Oddly, the oval egg cell occupies nearly all the space in a cocoon, leaving little if any room for albumen therein (it may be extra-cocoonal). According to Kruglov and Frolenkova (1981), *Valvata* "chalazae" have species-specific morphologies (grooves, swellings, etc.) — which is not the case with any other CHae known to me. Houbrick (1981) observed and reported but did not illustrate *Campanile* "chalazae". He expressed doubts about their being true CHae. Thus the homologies of both *Valvata* and *Campanile* "chalazae" are uncertain.

CHae-like structures occur in some of the freshwater ("higher limnic") Basommatophora. Bondesen (1950) reported "egg strings (*fila ovi*)" in egg masses of the Physidae and Lymnaeidae; Régondaud (1973) reported them also in the Chilinidae. In Bondesen's complex terminology, these strings are extensions of the tertiary envelope of the 2nd order, or the external membrane, and are "built up of several layers of fine lamellae" (Bondesen, 1950:78). The egg strings described and illustrated by Bondesen are indistinct, and he seems wrongly to have homologized them with CHae. The differences are best seen by comparing Bondesen's figure 35A and C, showing *Radix* (lymnaeid) egg cocoons with egg strings (f.o.) and Pelseneer (1914: fig. 5).

HETEROSTROPHY (HET)

Heterostrophy is hyperstrophic coiling of just the larval shell. Hyperstrophy has been defined, illustrated, and discussed most clearly by Knight (1952). Gastropod coiling can be likened to a spiral spring before and after it has been pulled through itself. Before, it is called orthostrophic; after, it is hyperstrophic; planispirality (coiling almost in one plane) is the intermediate condition. The direction of coiling *around* the axis and anatomical asymmetry are unreversed; it is the *polarity* of the axis that

Table 3. Systematic list of gastropods hypothesized to have homologous ciliated strips (CSs) in their mantle cavities.

Taxon	Term applied (key words)	Reference
PROSOBRANCHIA		
Mesogastropoda		
Superfamily uncertain		
Omalogyridae?		
Omalogyra? [Note 8]	strips of ciliated columnar cells (epithelium)	Fretter, 1948
Rissoellidae?		
Rissoella? [Note 8]	strips of columnar ciliated cells (epithelium)	Fretter, 1948
Architectonicacea		
Architectonicidae		
Heliacus	ciliated tracts (dorsal crest)	Robertson, 1974
Heliacus	ciliary tracts	Haszprunar, 1985b
Pyramidellacea		
Pyramidellidae	ciliated ridges; strips	Fretter and Graham, 1962
Boonea	ciliated tracts	Robertson, 1974
"Chrysallida"	strip of ciliated epithelium (cells)	Fretter and Graham, 1949
Odostomia	ciliated strips on ridges	Fretter and Graham, 1949
Pyramidella	CSs	Robertson, unpublished
Turbonilla	strip of ciliated columnar epithelium	Fretter, 1951
Turbonilla	ciliated strip	Fretter and Graham, 1962
Turbonilla 3 spp.	CSs	Robertson, unpublished
OPISTHOBRANCHIA ("Euthyneura" in part)		
Bullomorpha ("Cephalaspidea")		
Acteonidae		
Acteon	raphé; bande ciliée; bandelette ciliée	Perrier and Fischer, 1911
Acteon	ciliated ridge	Fretter and Graham, 1954
Acteon	dorsal and ventral ciliated bands	Fretter, 1975
Acteon	ridges	Brace, 1977
Maxacteon	raphae	Rudman, 1972c
Pupa	ciliated ridges; raphae	Rudman, 1972c
Bullinidae		
Bullina	ciliated ridges; raphae	Rudman, 1972a
Hydatinidae		
Hydatina	raphé; bande ciliée	Perrier and Fischer, 1911
Hydatina	raphae	Rudman, 1972b
Micromelo	ciliated ridges; raphae	Rudman 1972a
Ringiculidae		
Ringicula	ciliated band	Fretter, 1960
Bullidae		
Bulla	ciliated bands; ridge	Brace, 1977
Atyidae		
Haminoea	raphés palléaux; bande épithéliale ciliée	Perrier and Fischer, 1910
Haminoea	ciliated ridges; raphae	Rudman, 1971
Haminoea	ciliated bands; ridge	Brace, 1977
Phanerophthalmus	raphae	Rudman, 1972e
Smaragdinella	raphae	Rudman, 1972e
Scaphandridae		
Cylichna	raphae	Lemche, 1956
Scaphander	raphé; bande épithéliale, etc.	Perrier and Fischer, 1911
Scaphander	superior raphe	Thompson, 1976
Scaphander	ciliated bands, ridge	Brace, 1977
Akeridae		
Akera	raphés palléaux, etc.	Perrier and Fischer, 1911
Akera	raphes; tracts	Morton, 1972
Akera	ciliated bands; ridge	Brace, 1977
Aglajidae		
Aglaja	ciliated bands; ridge	Brace, 1977
Chelidonura	dorsal and ventral ciliated stripe	Marcus, 1955

Table 3 (continued)

Taxon	Term applied (key words)	Reference
Philinidae		
Philine	raphae	Rudman, 1972d
Philine	ciliated bands; ridge	Brace, 1977
Aplysiomorpha ("Anaspidea")		
Aplysiidae?		
Aplysia? [Note 9]	patch of long cilia	Marcus and Marcus, 1957
Ascoglossa ("Sacoglossa")		
Juliidae		
Berthelinia	ciliated ridges	Kay, 1968
Volvatellidae		
Volvatella	ciliated ridges	Kay, 1968
Oxynoidae		
Oxynoe	ciliated ridges	Kay, 1968
Cylindrobullidae		
Cylindrobulla	ciliated ridges	Marcus and Marcus, 1956
PULMONATA ("Euthyneura" in part)		
Basommatophora		
Amphibolidae		
Amphibola	ciliated band	Pilkington *et al.*, 1984
Salinator	Flimmerband; Cilienband	Hubendick, 1945
Siphonariidae		
Siphonaria	bandelettes ciliées	Dieuzeide, 1935
Siphonaria	Flimmerband; Cilienband	Hubendick, 1945
Siphonaria	ridge	Yonge, 1952
Siphonaria	ciliated pallial ridges; etc.	Marcus and Marcus, 1960
Siphonaria	dorsal and ventral ciliated bands	Fretter, 1975
Siphonaria	ciliated ridge	Hubendick, 1978
Williamia	Cilienband; Flimmerband	Hubendick, 1945
Chilinidae		
Chilina	Flimmerleiste	Haeckel, 1911
Chilina	Flimmerband; Cilienband	Hubendick, 1945
Chilina	pulmonary cavity lamella	Harry, 1964
Chilina	dorsal and ventral ciliated bands	Fretter, 1975
Chilina	ciliated bands	Brace, 1983
[Latiidae? *Latia*? Note 10]		
[Planorbidae? Note 11]		

changes. Thus the base of the shell grows into a false spire, and *vice versa*. Dextral and hyperstrophic shells, with the false spire directed upwards, have left-handed shells that commonly are called sinistral (e.g. Vermeij, 1975, who did not distinguish the five gastropod coiling types). I prefer to use the term "sinistral" for *anatomical* mirror image reversals of symmetry, *i.e.* reversals in the direction of coiling *around* the axis. Anatomical sinistrality is already established early in embryology (Crampton, 1894) and cannot be reversed during the life of a gastropod. Thus a sinistral gastropod cannot suddenly become dextral, but a hyperstrophic (HETic) one can, and commonly does, become orthostrophic. This happens at metamorphosis in many of the taxa listed in Table 5. The functional significance of HET is unknown, and its presence in so many disparate taxa is remarkable.

As can be seen from Table 5, HET is common in many groups of opisthobranchs and their presumed prosobranch and pulmonate relatives. Strictly speaking, though, they are not all HETic. One usually thinks of a HETic protoconch at the tip of a teleoconch. The thecosome families Limacinidae and Peraclidae are hyperstrophic and remain so throughout life. They are holoplanktonic and do not really metamorphose; essentially, they are neotenous larvae (Bandel *et al.*, 1984). There are also the ascoglossan and nudibranch slugs, which discard their larval shells soon after metamorphosis and do not grow a teleoconch. By extension, these cases are grouped with HET in this paper. Another taxon interesting from the standpoint of coiling is *Blauneria* (Ellobiidae), which is anatomically sinistral and has a HETic (right-handed) protoconch.

"Anastrophie" and "co-axial heterostrophy" are terms introduced, respectively, by Dautzenberg and Fischer (1896) and Minichev and Starobogatov (1971) for the kind of HET seen in low-spired, widely umbilicate shell forms such as architectonicids and *Cyclostremella*,

Table 4. Systematic list of gastropods hypothesized to have homologous chalazae (CHae) in their egg capsules and masses.

Taxon	Term applied (key words)	Reference
PROSOBRANCHIA		
Mesogastropoda		
Valvatacea?		
Valvatidae?		
Valvata?	Chalazen; Faden	Nekrassow, 1928a, b
Valvata?	thread; string; cord	Cleland, 1954
Valvata?	chalaza	Fretter and Graham, 1962
Valvata? [see text]	[chalazae]	Kruglov and Frolenkova, 1981
Cerithiacea?		
Campanilidae?		
Campanile? [see text]	chalazae	Houbrick, 1981
Architectonicacea		
Architectonicidae		
Architectonica	string	Bandel, 1976a
Heliacus cylindricus, H. perrieri	CHae	Robertson, unpublished
Philippia	chalazae	Robertson, 1970
Mathildidae?		
Gegania?	chalazae [Note 12]	Climo, 1975
Pyramidellacea		
Pyramidellidae		
Odostomia	mucus string	Rasmussen, 1944
Odostomia	mucus string; chalaza	Rasmussen, 1951
"*Odostomia*"	fine canal	Amio, 1959
"*Odostomia*"	[thin flagellate appendage]	Minichev, 1971
Odostomia	[thick cord-chalaza]	Minichev, 1971
Odostomia	chalazae	Robertson, 1976
OPISTHOBRANCHIA ("Euthyneura" in part)		
Bullomorpha ("Cephalaspidea")		
"Acteocinidae"		
Acteocina	chalazal material	Mikkelsen and Mikkelsen, 1984
Aglajidae		
Aglaja	connections . . . ; string	Bandel, 1976b
Aglaja	[CH]	Fasulo *et al.*, 1982
Philinidae		
Philine	threads	Horikoshi, 1967
Aplysiomorpha ("Anaspidea")		
Aplysiidae		
Aplysia	thin thread	Fujita, 1904
Aplysia	fine filaments	Amio, 1955
Pleurobranchomorpha? ("Notaspidea")		
Pleurobranchidae?		
Berthellina?	short tube or tiny capsule [Note 13]	Bandel, 1976b
PULMONATA ("Euthyneura" in part)		
Basommatophora		
Ellobiidae		
Ellobium	gelatinous threads	Ewald, 1963
Ellobium	[CHae]	Marcus and Marcus, 1963
Ellobium	chalaziform filaments	Berry *et al.*, 1967
Ellobium	mucoid threads	Sumikawa, 1983
Melampus	allochalazal strand	Russell-Hunter *et al.*, 1972
Ovatella	chalaziform filament	Morton, 1955c
Amphibolidae		
Salinator	[CHae]	Tanaka, 1959
Siphonariidae		
Siphonaria	fine string	Hutton, 1882
Siphonaria	[CHae]	Fujita, 1895

Table 4 (continued)

Taxon	Term applied (key words)	Reference
Siphonariidae (continued)		
Siphonaria	fine threads	Fujita, 1904
Siphonaria	fine threads	Abe, 1940
Siphonaria	cord of gelatinous substance	Abe, 1941
Siphonaria	strings	Borland, 1950
Siphonaria	fine filaments	Amio, 1955
Siphonaria	fine canal	Amio, 1959
Siphonaria	fine thread	Voss, 1959
Siphonaria	[CHae]	Marcus and Marcus, 1960
Siphonaria	fino ligamento	Olivier and Penchaszadeh, 1968
Systellommatophora		
Onchidiidae		
Onchidella	funicule	Joyeux-Laffuie, 1882
Onchidella	fine strand	Fretter, 1943
Onchidium	cordon	Serène, 1937
Onchidium	gelatinous knot; cords	Awati and Karandikar, 1948
Veronicellidae	processus chalaziformes	Franc, 1968 [Note 14]

a near planispiral pyramidellid. These authors mistakenly believed that the axes of protoconch and teleoconch coiling coincide. Except in architectonicids with planispiral larval shells, the axes do not coincide: see my drawing of this (Robertson, 1976). In pyramidellids there can be suppressed HET, with the larval shell consisting of only about one nearly planispiral whorl, in which case the protoconch and teleoconch axes probably do coincide approximately. But the direction in which the false spire of a *several-whorled* HETic protoconch projects correlates with (is determined by?) the shape of the teleoconch, specifically whether it has a wide umbilicus. If there is no wide umbilicus (as in mathildids, and nearly all pyramidellids) the false spire has to project transversely (protoconch resorption seeming not to occur). Since they intergrade with transverse HET, the terms anastrophy and its synonym co-axial HET seem superfluous.

The three other characters are usually constant in their presence or absence in a genus or family. HET, on the other hand, commonly is sporadic within a genus (or species?). In certain European species of *Odostomia*, Thorson (1946) and Rodriguez Babio and Thiriot-Quiévreaux (1975) believed that planktotrophy correlates with HET, and lecithotrophy with reduced or wholly suppressed HET. This generality is not true of certain east North American species of *Fargoa (Odostomia, sensu lato)* that combine planktotrophy and suppressed HET (Robertson, unpublished). Suppressed HET may relate more to phylogeny than larval ecology. In the two genera in the Amphibolidae, both of which are planktotrophic, monotypic *Amphibola* is HETic while at least one species of *Salinator* is wholly orthostrophic (Pilkington and Pilkington, 1982; Tanaka, 1959). These examples show that HET cannot safely be predicted from planktotrophy in HET-prone taxa, and *vice versa*. However, it is possible that HET and lecithotrophy never go together.

HET has been reported in various prosobranchs not in Table 5. Minichev (1971) wrote that HET is apparently a widespread phenomenon among Prosobranchia. I disagree. The supposedly HETic taxa include the Trochidae, Docoglossa, Cyclophoridae, Rissoidae, Turritellidae, Aclididae, Eulimidae, Epitoniidae (Note 15), Janthinidae, Calyptraeidae, and Muricidae. It is beyond the scope of this paper to confirm or refute these mainly undocumented reports.

DISCUSSION AND CONCLUSIONS

The four characters treated herein co-occur in two families combining some prosobranch with some opisthobranch traits. These are the Architectonicidae and Pyramidellidae, both classified as prosobranchs in the present paper. Two more prosobranch families, also with some opisthobranch traits, the Epitoniidae and Janthinidae, have well-developed larval and postlarval PMOs, but lack CSs, CHae, and probably HET. The four characters are otherwise unknown in prosobranchs except for a few doubtful cases.

Among opisthobranchs, all four characters occur in the Bullomorpha, which is the most "primitive" order in this diverse subclass. In other opisthobranch orders, the four characters do not co-occur. Indeed, their patterns of taxonomic occurrence appear irregular. In the Aplysiomorpha and Ascoglossa, three of the characters are represented in each; in the Pleurobranchomorpha, two; in the Thecosomata, Acochlidiacea, and Nudibranchia, one each, and in the highly specialized Gymnosomata, none.

Larval PMOs are known in the Bullomorpha, Pleurobranchomorpha, and Ascoglossa. In the Bullomorpha, certain species of *Retusa* and *Philine* are the only opisthobranchs known to retain PMOs past me-

Table 5. Systematic list of Recent gastropods hypothesized to have homologous heterostrophy (HET), including hyperstrophy of some adult thecosomes, and hyperstrophy of some larval ascoglossan and nudibranch slugs.

Taxon	Term applied (key words)	Reference
PROSOBRANCHIA		
Mesogastropoda		
Epitoniacea? ("Ptenoglossa")		
Epitoniidae?		
Couthouyella? [Notes 2, 15]	HET	Gosliner, 1981
Architectonicacea		
Architectonicidae	HET [Note 16]	Robertson, 1963b
Architectonica	protoconch . . . sinistral	Hedley, 1903
Architectonica	sinistral	Bandel *et al.*, 1984
Heliacus	Protoconch . . . hyperstroph	Bieler, 1984c
Heliacus spp.	HET	Robertson, unpublished
Philippia	[HET]	Pelseneer, 1888a
Philippia	hyperstrophic larval shells	Robertson, 1964
Philippia	hyperstrophic protoconchs	Robertson, 1970
Philippia	hyperstrophically coiled larval shell	Robertson *et al.*, 1970
Philippia	hyperstrophically coiled protoconch	Robertson, 1976
Pseudomalaxis	[slight HET]	Hedley, 1903
Pseudomalaxis	hyperstropher Protoconch	Bieler, 1984a
Pseudomalaxis	Protoconch anastroph	Bieler, 1984b
Mathildidae		
Gegania	heterostrophe protoconch	Climo, 1975
Mathilda	heterostrophe . . . apex	Jeffreys, 1884
Mathilda	[HET]	Hedley, 1903
Pyramidellacea		
Pyramidellidae [incl. Cyclostremellidae; Note 5] [Note 17]		
Angustispira	nucléus sénestre	Pelseneer, 1912
Boonea	sinistral embryonic shell	Wells, 1959
Boonea	[HET]	Wells and Wells, 1961
Boonea	HET	Robertson, unpublished
Cyclostremella	heterostrophic protoconch	Moore, 1966
Cyclostremella	hyperstrophic protoconch	Robertson, 1973
Cyclostremella	coaxial heterostrophy	Hickman, 1980
Ebala	sinistral larval shell	Rasmussen, 1944
Myxa	heterostrophe apex	Hedley, 1903
"*Odostomia*"	nucléus sénestre	Pelseneer, 1912
Odostomia	sinistral	Rasmussen, 1944
Odostomia	sinistral	Rasmussen, 1951
Odostomia	hyperstrophically coiled protoconch	Robertson, 1976
Pseudoskenella	slight . . . heterostrophy	Ponder, 1974
Sayella [family?]	sinistral nucleus	Dall, 1885
Turbonilla	[HET]	Hedley, 1903
OPISTHOBRANCHIA		
Bullomorpha ("Cephalaspidea")		
Acteonidae		
Acteon	[HET]	Thorson, 1946
Acteon	heterostrophy	Lemche, 1948
Diaphanidae		
Diaphana	[HET]	Thorson, 1946
Diaphana	heterostrophy	Lemche, 1948
"Acteocinidae"		
Acteocina	nucléus . . . sénestre	Dautzenberg and Fischer, 1896
Acteocina	hyperstrophic . . . protoconch	Mikkelsen and Mikkelsen, 1984
Retusidae		
Retusa	sinistral	Rasmussen, 1944
Retusa	[HET]	Thorson, 1946
Retusa	heterostrophy	Lemche, 1948

Table 5 (continued)

Taxon	Term applied (key words)	Reference
Scaphandridae		
Cylichna	heterostrophy	Lemche, 1948
Roxania	heterostrophy	Lemche, 1948
Scaphander	heterostrophy	Lemche, 1948
Akeridae		
Akera	[HET]	Thorson, 1946
Akera	heterostrophy	Lemche, 1948
Philinidae		
Philine	[HET]	Thorson, 1946
Philine	heterostrophy	Lemche, 1948
Philine	hyperstrophy	Horikoshi, 1967
Thecosomata		
Limacinidae		
Limacina	ultra-dextres	Pelseneer, 1888b, 1891
Limacina	[HET]	Lemche, 1948
Limacina	sinistral	Morton, 1954a
Limacina	[HET]	Rampal, 1964
Limacina	sinistral	Bandel *et al*., 1984
Peraclidae		
Peracle	ultra-dextres	Pelseneer, 1888a, b, 1891
Peracle	sinistral	Bandel *et al*., 1984
Cymbuliidae		
Cymbulia	ultra-dextres	Pelseneer, 1888a
Gleba	sinistral	Pelseneer, 1888a
Aplysiomorpha ("Anaspidea")		
Aplysiidae		
Aplysia	[HET]	Strenth and Blankenship, 1978
Pleurobranchomorpha ("Notaspidea")		
Pleurobranchidae	[HET]	Gohar and Abul Ela, 1957; Thompson, 1961, shell-type 1
Acochlidiacea		
Hedylopsidae		
Hedylopsis	[HET]	Swedmark, 1968: fig. 2H
Ascoglossa ("Sacoglossa")		
Several families	[HET]	Thompson, 1961, shell-type 1
Oxynoidae		
Oxynoe	[HET]	Hamatani, 1980
Stiligeridae		
Stiliger	[HET]	Rasmussen, 1951
Elysiidae		
Elysia	[HET]	Thorson, 1946
Stiligeridae		
Stiliger	[HET]	Rasmussen, 1951
Limapontiidae		
Limapontia	[HET]	Thorson, 1946
Nudibranchia		
Many families [Note 18]	[HET]	Thompson, 1961, shell-type 1; Hurst, 1967; Soliman, 1977, shell type B
PULMONATA		
Basommatophora		
Ellobiidae ("Melampidae") [Note 19]		
Blauneria	renversement des tours embryonnaires	Fischer and Crosse, 1886?
Blauneria	[larval] shell . . . dextral [sinistral anatomy]	Marcus and Marcus, 1963
Detracia	nucleus . . . inverted	Dall, 1885
Detracia	[HET]	Marcus and Marcus, 1965
Ellobium	[newly] hatched . . . shell . . . sinistral	Marcus and Marcus, 1963
Leucophytia	reduced heterostrophy	Morton, 1955c
Marinula	inverted nucleus	Dall, 1885

continued

Table 5 (continued)

Taxon	Term applied (key words)	Reference
Ellobiidae (continued)		
Melampus	hétérostrophe	Fischer and Crosse, 1886?
Melampus	heterostrophic	Marcus and Marcus, 1965
Melampus	heterostrophy	Russell-Hunter *et al.*, 1972
Ovatella	reflexed apex	Clark, 1855 [Note 20]
Ovatella	heterostropher	Meyer, 1955
Ovatella	very reduced hyperstrophy	Morton, 1955c
Pythia	heterostrophic	Harry, 1951
Otinidae		
Otina	reduced heterostrophy	Morton, 1955a
Amphibolidae [Note 21]		
Amphibola	heterostrophy	Pilkington and Pilkington, 1982
Siphonariidae		
Siphonaria	sinestrally [sic]	Voss, 1959
Siphonaria	reduced heterostrophy	Marcus and Marcus, 1960
Williamia	Heterostrophie	Hubendick, 1945
Chilinidae		
Chilina	heterostrophic	Régondaud, 1973

PROSOBRANCHIA MESOGASTROPODA (some families)	Pigmented mantle organ in veliger	Pigmented mantle organ in postlarva	Ciliated strips	Chalazae	Heterostrophy
Valvatidae		—	—	?	—
Omalogyridae			?		—
Rissoellidae		?	?	—	—
Campanilidae			—	?	—
Epitoniidae	●	●	—	—	?
Janthinidae	●	●	—	—	—
Architectonicidae	●	●	●	●	●
Mathildidae		?	—	?	●
Pyramidellidae	●	●	●	●	●

Fig. 2. Taxonomic occurrences of the four characters in certain families of the subclass Prosobranchia, compiled from Tables 1, 3-5. A spot means that the character is known in one or more species in the family. Dashes mean that a character is probably absent from the family. Question marks denote doubtful cases, and blanks mean no information is available.

tamorphosis into the juvenile stage. CSs occur in the Bullomorpha, Aplysiomorpha (known in juveniles of one species only), and Ascoglossa (certain shelled forms only). Loss of postlarval PMOs and CSs would appear to result from reduction or loss of the shell and mantle cavity. CHae are known only in the Bullomorpha and Aplysiomorpha. HET (including postlarval holoplanktonic hyperstrophy and hyperstrophic larval shells of slugs) is the character most widespread among opisthobranchs: only the Gymnosomata lack HET (Lalli and Conover, 1976).

The four characters occur in the Pulmonata only in the "lower" (as opposed to "higher limnic") Basommatophora and Systellommatophora. Morton (1955b, c) believed the Ellobiidae to be the most "primitive" pulmonate, and the one most likely to have given rise to the

Stylommatophora. The Amphibolidae and Siphonariidae each have three out of the four characters; the Ellobiidae and Chilinidae, two each; the Otinidae, Onchidiidae and Veronicellidae, one each. A larval PMO occurs doubtfully in one of the two genera of the Amphibolidae. CSs occur in the Amphibolidae, Siphonariidae, and Chilinidae. CHae occur in the Ellobiidae, Amphibolidae, Siphonariidae, Onchidiidae, and Veronicellidae. HET, again the most ubiquitous character, occurs in the Ellobiidae, Otinidae, Amphibolidae, Siphonariidae, and Chilinidae.

The co-occurrences of all four characters in the Architectonicidae, Pyramidellidae, and Bullomorpha support the ideas that, among themselves, these characters are homologous, and that these three taxa are related — although they deserve suprafamilial (or higher ranking) separation. In other taxa the concordances are not as good, but there seem also to be homologies and relationships that cross the subclass boundaries.

What other characters might have similar patterns of taxonomic occurrence? Simultaneous hermaphroditism and euthyneury are among the main characters traditionally used to distinguish euthyneurans from prosobranchs. Euspermatozoon morphology has also been used, and Morton (1955b, c) advocated use also of characters in the digestive, excretory, and reproductive

		Pigmented mantle organ in veliger	Pigmented mantle organ in postlarva	Ciliated strips	Chalazae	Hetero-strophy
OPISTHOBRANCHIA (all orders)	Bullomorpha	●	●	●	●	●
	Thecosomata		—	—		●
	Gymnosomata		—	—		—
	Aplysiomorpha	—	—	?	●	●
	Pleurobranchomorpha	●	—		?	●
	Acochlidiacea		—	—		●
	Ascoglossa	●	—	●	—	●
	Nudibranchia	?	—	—	—	●

Fig. 3. Taxonomic occurrences of the four characters in all eight orders in the subclass Opisthobranchia. Other conventions as for Fig. 2.

			Pigmented mantle organ in veliger	Pigmented mantle organ in postlarva	Ciliated strips	Chalazae	Hetero-strophy
PULMONATA (some families)		Ellobiidae	—	—	—	●	●
	BAS	Otinidae	—	—	—	—	●
		Amphibolidae	?	—	●	●	●
		Siphonariidae	—	—	●	●	●
		Chilinidae		—	●	—	●
	SYS	Onchidiidae	—	—	—	●	—
		Veronicellidae			—	●	—

Fig. 4. Taxonomic occurrences of the four characters in certain families of the subclass Pulmonata. BAS = Basommatophora; SYS = Systellommatophora. Other conventions as for Fig. 2.

systems. Other potentially useful characters include radulae and spermatophores, although each of these may be especially prone to converge.

There are two main problems with simultaneous hermaphroditism as a taxonomic character: 1, it intergrades with protandry, and 2, it has evolved repeatedly in mesogastropods. Except for some dioecious Acochlidiacea (Odhner, 1938; Marcus, 1953; Marcus and Marcus, 1954), simultaneous hermaphroditism supposedly occurs throughout the two euthyneuran subclasses. Often, though, male gametes mature first. True simultaneous hermaphroditism, with substantial overlap of the two sexes, seems to occur in one or more species in the following mesogastropod families: Viviparidae (Alonte, 1930), Valvatidae (Cleland, 1954; Fretter and Graham, 1962, but see Furrow, 1935), Rissoellidae (Fretter, 1948), Lamellariidae (Fretter and Graham, 1962), Eulimidae *sensu lato* (Ponder and Gooding, 1978, and references therein; Fretter and Graham, 1982), Architectonicidae (Robertson, 1974), and Pyramidellidae (Fretter and Graham, 1949). There is diverse sexuality even within the Eulimidae (Warén, 1980a).

Gosliner (1981) has explained how euthyneury in some cases is distinguished from streptoneury with difficulty, and how pyramidellid euthyneury may have had a different origin from non-pyramidellid euthyneury. This is noteworthy because if pyramidellids are prosobranchs, they apparently are the only taxon with euthyneury in the subclass (Fretter and Graham, 1949, 1962). Fretter and Graham (1954, 1962) and Gosliner (1981) have also discussed the retention of streptoneury in certain bullomorph opisthobranchs (such as the Acteonidae, Diaphanidae (*Toledonia*), Ringiculidae, and Scaphandridae), and the occurrence of streptoneury and euthyneury even in the same family (Diaphanidae) or genus *(Acteocina)* (Gosliner, 1979). Thus streptoneury and euthyneury are not good either for separating gastropod higher taxa.

Morphologically, the euspermatozoa of the Pyramidellidae are more like those of euthyneurans than they are of mesogastropods (Franzén, 1955; Thompson, 1974). So also are the euspermatozoa of an architectonicid (Healy, 1982) and a stenothyrid rissoacean (Healy, 1983). Healy suggested that the latter case might be convergent. Few "lower" mesogastropod euspermatozoa have so far been adequately studied, and it seems more likely that morphological diversity is greater than currently appreciated and that euthyneuran traits occur among some of them.

The most "primitive" representatives of both euthyneuran subclasses (bullomorphs and basommatophorans) are similar to one another anatomically and functionally; they must have shared one or more prosobranch ancestors (Boettger, 1955; Morton, 1955b, c). Which group or groups of prosobranchs were ancestral to euthyneurans? On the basis of radular and other anatomical characters, Hubendick (1945) argued for a diotocardian (lower archaeogastropod) ancestor. However, Graham (1949) and Morton (1955b) have given compelling reasons for rejecting this idea. Instead, basing his conclusions mainly on characters of the digestive and reproductive systems, Morton (1955b:159) proposed a monotocardian origin for pulmonates, suggesting "an origin ... much lower than any modern mesogastropod." After all, "we are not ... likely to find among living families any of the stages in a sequence that must have been completed early [late] in the Paleozoic" *(Ibid.)*. Earlier, Morton (1954b:197) had believed protandry to be primitive in gastropods and that pulmonates and opisthobranchs "probably originated close together at a higher archaeogastropod level and have retained their hermaphroditism." Boettger (1955) and Fretter and Graham (1962) believed that opisthobranchs arose from rissoaceans or cerithiaceans. Marcus and Marcus (1965) stated that the stomachs of certain ellobiids and a siphonariid "contain pre-mesogastropodan traits." Fretter (1980) believed in a "procerithiacean" origin of euthyneurans. Gosliner (1981) has presented data and arguments supporting an early littorinacean origin. According to Healy (1983:212), "it would be difficult ... to derive euthyneuran sperm features from euspermatozoa of *Littorina* species that have been studied."

The Epitoniacea, Architectonicacea, and Pyramidellacea are "higher" mesogastropod superfamilies that are highly specialized — especially as regards their postlarval feeding biology. Epitoniids and architectonicaceans both have acrembolic proboscises and other features of the digestive system adapted for feeding on coelenterate body tissues; pyramidellaceans also have acrembolic proboscises, but these are used for feeding suctorially on the body fluids of miscellaneous invertebrates. How could these gastropods revert to detritivory, herbivory, and the variety of other modes of feeding found in euthyneurans (Graham, 1955)? For these and other reasons, it seems most unlikely that any of these acrembolic proboscis-bearing prosobranchs gave rise to euthyneurans even in the distant past. The ancestor or ancestors of euthyneurans seem to have had a mosaic of traits now retained in prosobranchs only among certain "lower" mesogastropods and in the three "higher" mesogastropod superfamilies given special attention in this paper. Thus the origin of opisthobranchs is an excellent example of mosaic evolution (Stanley, 1979).

NOTES APPENDED TO TABLES

1. The pigmented (and in one species multiple) "blotches" in postlarval *Rissoella* (Fretter, 1948) seem doubtfully to be PMOs. The "pigmented hypobranchial gland" in *Omalogyra* (Fretter and Graham, 1962:639) appears to have been a *lapsus* for *Rissoella*.
2. Apparently independently, Warén (1980b) and Gosliner (1981) transferred *Couthouyella* to the Epitoniidae.
3. The larval *"Aclis"* (Aclididae) with a "probable excretory organ" is probably an *Epitonium* (Richter and Thorson, 1975).
4. This organ is on the animal's left side and Climo believed it to be either respiratory or an anal gland.

5. Robertson (1973) synonymized the Cyclostremellidae with the Pyramidellidae.

6. The postlarval "pallial gland" of *Philinoglossa* may have been wrongly homologized with a PMO by Salvini-Plawen (1984). This posterior, median and colored gland next to the anus has been studied by Marcus (1953). This gland does not resemble a PMO histologically.

7. This presumably is shown unlabeled above the midline in figure 2A. Since this is the only report of a pigmented "larval kidney" in the Basommatophora, it needs to be described and better illustrated before the report is accepted. Farnie (1924) studied newly hatched veligers of the same species, and neither illustrated nor described such an organ. PMOs generally are present already in the newly hatched veliger. Tanaka (1959) illustrated a full-grown *Salinator* veliger. Again, no such organ is illustrated. For these reasons I question the occurrence of PMOs in amphibolids and other basommatophorans. (I have read the literature on ellobiid, chilinid, otinid, trimusculid, siphonariid, and onchidiid veligers.)

8. *Omalogyra* and *Rissoella* CSs are doubtfully homologous because they arise posteriorly at the anus, somewhat anterior to the mantle cavity hind end.

9. Observed only in a 2 mm long juvenile.

10. CSs in *Latia* (Latiidae) have been mentioned by Haeckel (1911), Hubendick (1945), and Harry (1964). I have been unable to trace a description or illustration even in Pelseneer (1901).

11. The "pulmonary cavity lamella" of the Planorbidae (including Bulinidae) may be homologous to the *Chilina* CS (Harry, 1964).

12. The cocoon is shown ill-defined, and at least the beginning of the upper "chalaza" is unusually thick, making it a doubtfully homologous CH.

13. The swellings make this CH anomalous and doubtfully homologous.

14. Original source not traced.

15. According to Gosliner, *Couthouyella* has a HETic protoconch — which is otherwise unknown in the Epitoniidae. Bartsch (1909:110) reported that the type-species has a "Nucleus ... of about one and one quarter ... dextral turns." His figure (pl. 11, fig. 13) bears this out.

16. Some architectonicid larval shells are planispiral; otherwise, they are all HETic.

17. No attempt has been made here to canvas the extensive literature on pyramidellid HET. Some of the most biologically oriented papers are cited.

18. Hurst (1967) has shown how nudibranch type 1 larval shells grade from planispirality into slight and moderate hyperstrophy.

19. Dall (1885:275) records a (reduced) "sinistral nucleus" in *Carychium*. HET is not otherwise reported in this or any other land snail genus.

20. This appears to be the oldest published observation on HET.

21. It is puzzling that Tanaka (1959, figs. 8-9) did not observe or illustrate HET in a planktotrophic *Salinator*. See also Note 7 in this connection.

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POSTSCRIPT

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NOTES ON MOLLUSKS OF DEEP-SEA VENTS AND REDUCING SEDIMENTS

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ABSTRACT

Only four classes (Aplacophora, Bivalvia, Gastropoda and Cephalopoda) of the seven classes of mollusks have been recorded from the hydrothermal vents and the cold sulfide seeps. Comparisons are made between these faunas. The nine species of aplacophorans are all from the vents and are all predators on coelenterates and foraminifera. It is suggested that *Calyptogena magnifica* is unique among the Vesicomyidae in becoming epifaunal at the vents and that the Lucinacea and Solemyidae are constrained to soft sediment habitats by their method of feeding. The parallel evolution of the lucinids and the solemyids is discussed. A comparison of the taxonomic composition of the prosobranch snail faunas at the vents and a typical soft-bottom deep-sea environment shows striking differences, in particular, that the percent species composition of archaeogastropods and neogastropods is reversed - i.e. at the vents the archaeogastropods rather than the neogastropods are the predominant group. It is suggested that this may be attributed to the reduction of predators at the vents. Faunas of the newly discovered ambient temperature sulfide/hydrocarbon sites in the Gulf of Mexico (the Florida Escarpment and Louisiana Slope) are compared with those of the hydrothermal vents - the similarities are striking.

The discovery of the hydrothermal vents at the Galapagos Rift in 1977 came as no surprise to geologists. Work at the Galapagos Rift was a part of a geophysical program begun in 1974 (but with roots extending much further back) to study the mid-ocean ridges and the formation of the ocean floor. These studies led to the discovery of the incredible hot vents on the East Pacific Rise at 21°N and confirmation of the theory of plate tectonics (for review see Mottl, 1983; Ballard, 1984). Understanding the circulation of the water through the vent systems (where it reacts with the crustal rocks at very high temperatures, leaving behind some minerals and picking up others), has allowed chemical oceanographers to balance the chemistry of the ocean, a problem the answer to which had evaded chemists since the days of the Challenger Expedition (Edmonds and VonDamm, 1983).

It was the biological world that was taken completely by surprise with the discovery of the vents surrounded by incredible numbers of large clams, mussels and enormous vestimentiferan tube worms. (Phylum Pogonophora, subphylum Vestimentifera). These large organisms, along with the brachyuran and galatheid crabs, the enteropneusts, and limpets are the obvious inhabitants of the area, but the organisms which are at the heart of the system and the base of the food chain are the free-living and/or symbiotic chemolithoautotrophic (more commonly called chemoautotrophic, or chemosynthetic) bacteria. These bacteria can oxidize the sulfides emanating from the vents and utilize the energy to synthesize organic compounds from carbon dioxide (as land plants, algae and phytoplankton use sunlight as an energy source in photosynthesis). Bacteria in the water column and the varied bacterial mats that can be seen as a fine fuzz covering nearly everything in the immediate vent area are a source of nutrients for a variety of filter feeders and grazers. Free-living sulfur-oxidizing bacteria capable of chemosynthesis have been known since 1887 but, until the discovery of the hydrothermal vents where the bacteria were found to support large numbers of filter feeders and grazers, they were not considered ecologically important (Jannasch, 1984). Even more amazing was the discovery of the symbiotic association of these bacteria with the large tube worms, clams and mussels because chemosynthetic bacteria as symbionts within animal tissue was not known (Cavanaugh, 1983a, and in press). It was soon realized that this mode of nutrition was important to other mollusks living in sulfide-rich habitats (Cavanaugh, 1980, 1983b) and these findings opened up a whole new area of molluscan ecology. Chemosynthetic bacteria symbiotic with the vestimentiferans, clams and mussels are not only a nutrient source for these organisms but also through them for a large variety of grazers, scavengers, detritus feeders and predators.

Water issuing from and immediately surrounding the vents is not only rich in sulfides (the source of energy for primary production by the bacteria) but it is also highly toxic. The organisms living in the area must be able to tolerate or in some way detoxify these substances. These organisms and/or assemblages have come to be known as vent-type organisms and/or communities. When the emission of hydrothermal fluids ceases and the vent 'shuts down', the community collapses and scavengers move in from the 'outside' to take advantage of the fare.

Sulfide based communities, however, are not unique to hydrothermal vents. They may be found at ambient bottom temperatures in sulfide rich areas in both shallow and deep water. For example 1) a sulfide system comprised largely of meiofauna may be found in the black, sulfur-laden muds beneath the oxidized sandy bottom in shallow to deep waters (Fenchel and Reidl, 1970); 2) hypersaline, sulfide rich water may seep out onto the ocean floor from the base of escarpments at depths of 3000 m (Paull, Hecker, Commeau *et al*, 1984; Paull, Hecker, Neuman *et al*, 1984); or 3) may be found in regions of hydrocarbon seeps where gas and oil are rising to the surface of the sediments from deep hydrocarbon reservoirs beneath (Brooks, Kennicutt, Bidigare and Fay, 1985). Though at these ambient temperature sites the sulfides are largely within the substrata rather than in the water column, members of the macrofauna belong to the same major groups as those found at the vents - vestimentiferans, vesicomyids, mytilids - and these too apparently are dependent on symbiotic chemosynthetic bacteria. Primary production and the basic structure of the food chain is essentially the same whether the community is found at a hot vent or a cold seep. These sulfide-based communities may be found in relatively shallow to deep-sea wherever oxygen, sulfides, carbon dioxide and appropriate bacteria come together.

The purpose of these notes is to make some preliminary comparisons between the molluscan faunas of the hydrothermal vents and cold sulfide/hydrocarbon seeps (see Figure 1 for map showing the location of the known sites), to discuss some points that intrigue me but which I have not seen treated elsewhere, to point out how fragmentary our knowledge of these faunas really is, and by this means, stress the need for further observations and collecting.

Collections of mollusks from hydrothermal vents and seeps are, for the most part, inadequate for systematic work, let alone any meaningful statistical analysis. Consequently one can only indicate trends, point out possible relationships and suggest tentative directions for future investigations. Only four of the seven classes of Mollusca have been seen or collected at the hydrothermal vents and cold seeps - aplacophorans, bivalves, gastropods and cephalopods. No monoplacophorans, polyplacophorans, or scaphopods have, to my knowledge, been seen or collected. Cephalopods are rarely seen and none have been collected.

The Aplacophora from the vicinity of the vents (Juan de Fuca, East Pacific Rise at 21°N [US] and 12°-13°N [French], Galapagos Rift and Guaymas Basin) all have come from the washing of vestimentiferan tubes, clams and mussels, slurp gun samples, rock scrapings, crevice rubble and box cores. Amelie Scheltema (Woods Hole Oceanographic Institution) in a personal communication said that, from the immediate vent areas, she had received a total of 250 specimens, 94 from Juan de Fuca and 156 from the other sites, mostly Galapagos Rift and 21°N. Only six specimens were received from Guaymas Basin and four from 12°-13°N. From these collections she has identified nine new species representing five genera, two of which are monospecific and probably new, one from 12°-13°N, the other from Guaymas Basin. The genus *Simrothiella* Pilsbry 1898 (Family Simrothiellidae) is represented by a single new species at Juan de Fuca, three new species at 21°N, one shared with the Galapagos Rift; a second species at the Galapagos Rift is new and limited to the area. *Simrothiella* has not been found at 12°-13°N or at Guaymas Basin, but both sites have a new genus and species unique to the area. As with the limpets, the most diverse area is 21°N with four species—a new *Neomenia* Tullberg 1875 and three species of *Simrothiella*. Also, as with the limpets, one of these is shared with the Galapagos Rift. According to A. Scheltema these small predators (all Neomeniomorphs) are probably feeding on coelenterates growing on the vestimentiferan tubes, rocks and other solid surfaces. At Guaymas Basin a new species of *Falcidens* (a chaetodermatid) was taken in an ALVIN scoop on dive 1168. This species she believes probably feeds on foraminiferans. To date no aplacophorans have been recorded from any of the seeps.

For the bivalves and gastropods we are now beginning to accumulate sufficient data to make some comparisons and generalizations. The bivalve fauna of the vents and seeps is dominated by five families representing the three major gill types - protobranch (Solemyidae), filibranch (Mytilidae), and eulamellibranch (Lucinidae, Thyasiridae, and Vesicomyidae). Species at these habitats all harbor symbiotic bacteria in their gills and of these the vesicomyids and mytilids are among the most conspicuous organisms at the vents. Except for the archaeogastropod limpets, the gastropods (both mesogastropods and neogastropods) are generally small, inconspicuous and not abundant.

The vesicomyids (*Vesicomya* and *Calyptogena*) are deep-sea bivalves (Allen, 1983) and all species examined to date have large thick gills containing symbiotic, chemoautotrophic bacteria, and a short simple gut. It is possible that: 1) the vesicomyids are constrained by their dependence on the symbiotic bacteria to live in sulfide rich habitats and that the adaptation to this way of life arose only once in this family, or 2) the family is composed of covergent groups placed in one family, and the habit has evolved several times. The many genera and species now included in the Vesicomyidae have, by various authors, been included in the Carditidae, Veneridae, Kelliellidae and Cyprinidae [now Articidae]. The kelliellids are small, infaunal deep-sea bivalves whose shell characters, except for size, are indistinguishable from *Vesicomya* and Thiele (1934) included *Vesicomya* and *Calyptogena* in that family. It is possible that the large species found at the vents and seeps arose from the local kelliellids by adopting the habit of harboring symbiotic bacteria in the gills and, benefiting from the abundant food source, grew to a much larger size. The major adaptation that we see among the large white clams at the sulfide/hydrocarbon sites is the surface living, nestling

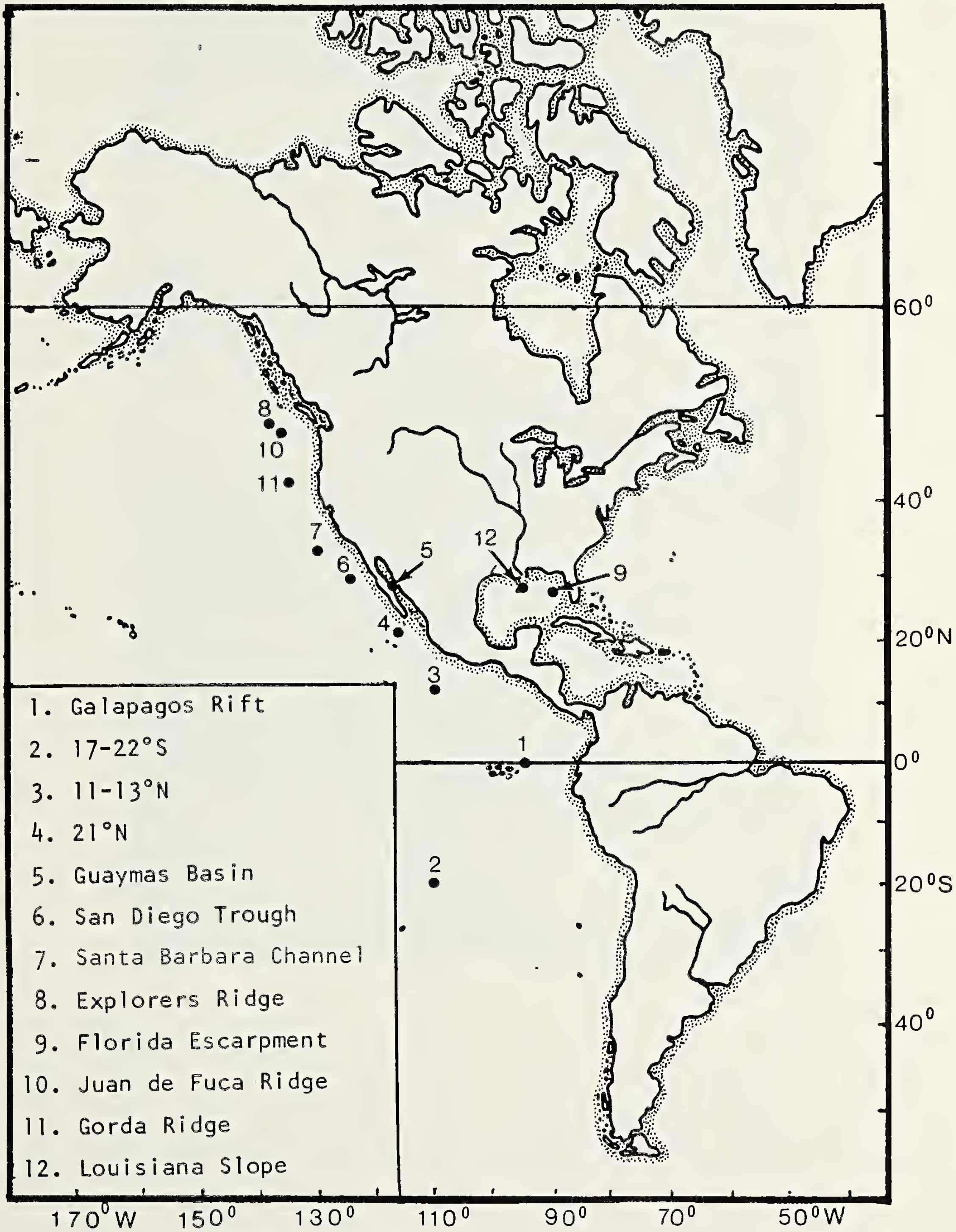

Fig. 1. Map showing location of the vents and seeps (adapted from *Oceanus* 27:9).

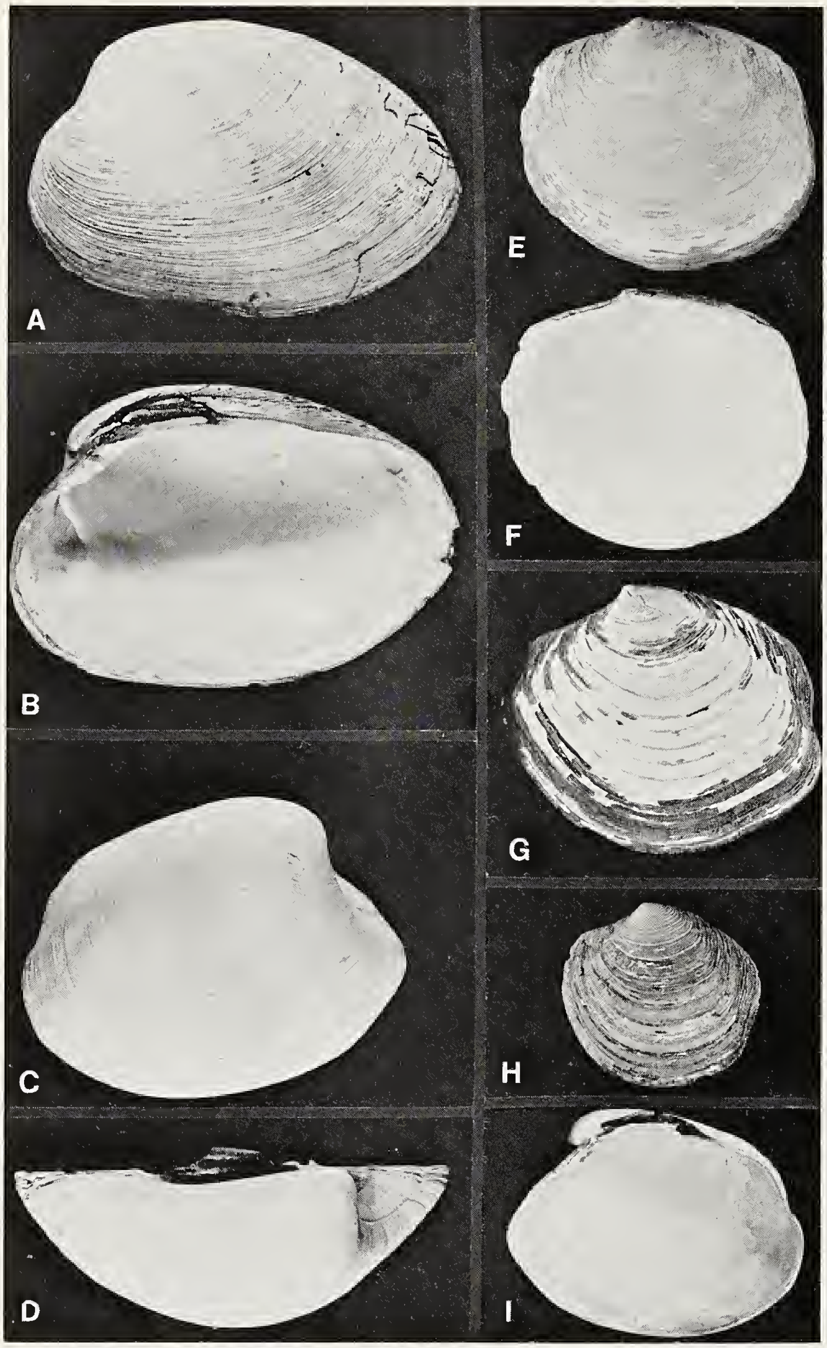

Fig. 2. Vesicomyids and lucinids from the Louisiana Slope (27° 45'N; 90° 14'W in 600-700m). A—B *Calyptogena ponderosa* Boss (actual length 90 mm). A. Outer view of left valve. B. Inner view of right valve showing hinge and ligament with the escutcheon posterior to it. C-D and I. *Vesicomya caudata* Boss (actual length 64 mm). C. Outer view of right valve showing the pronounced anteriorly inclined umbo and the lunule anterior to it. D. Dorsal view of right valve showing the dark ligament posterior to the umbo and the lunule anterior to it. I. Interior view of right valve showing size and position of the ligament, the hinge teeth and muscle scars. E-F. ?*Pseudomiltha* sp. (actual length 61.5 mm). E. Outer view of left valve showing small nearly central umbo and fine sculpture. F. Inner view of right valve showing long anterior adductor muscle scar, lack of hinge teeth and the shallow umbonal cavity of this compressed shell. G. *Lucina atlantis* McLean. Holotype MCZ 73345 (actual length 56 mm) from R/V Atlantis Station 1935, 38° 10'N; 75° 51'W in 300-118 fms. shown here for comparison with the specimen from the Louisiana Slope. H. *Lucinoma atlantis* (McLean). Specimen from the Louisiana Slope site (actual length 33 mm). Outer view of left valve showing the strong sculpture, the slightly concave dorsal margin anterior to the umbo and the ventrally sloping postero-dorsal margin. *Lucinoma atlantis* has been considered a synonym of *L. filosa* Stimpson and it may be but further comparative material and anatomical work is needed to prove this. There is no question that the specimens figured in G and H are the same species. (All photos by R. D. Turner.)

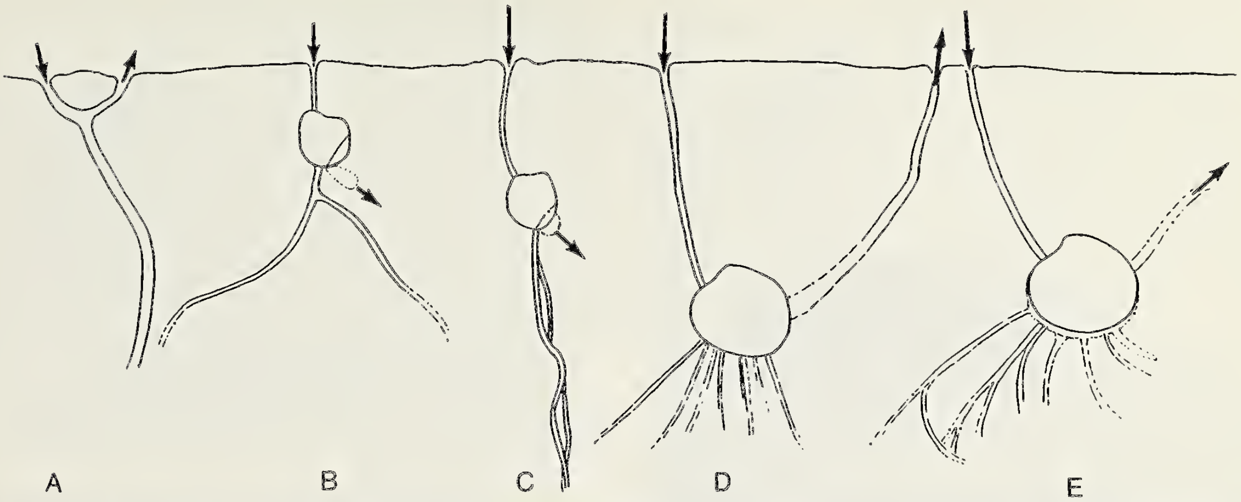

Fig. 3. Burrows of *Solemya* and species of Lucinidae as seen in Stanley's (1970) X-rays from which these sketches were made. A. *Solemya velum* Say from Mayaguez, Puerto Rico. B-C. *Lucina* [*Linga*] *pensylvanica* (Linne) from Biscayne Bay, Florida. The dotted areas posterior to the shells with excurrent arrows shows light in the X-rays and may indicate the excurrent area. D. *Lucina* [*Phacoides*] *pectinatus* (Gmelin) from Biscayne Bay, Florida. Like *Solemya* the excurrent tube reaches the surface. E. *Codakia orbicularis* (Linne), Biscayne Bay, Florida. The excurrent tube is directed upward but may not reach the surface. (Sketches after Stanley's X-rays by R. D. Turner.)

habit of the hard substratum, hydrothermal vent species, *Calyptogena magnifica* Boss and Turner 1980. Vesicomyids until the discovery of the vents were characterized as being infaunal and all vesicomyids found in the soft sediment sulfide/hydrocarbon sites are, in fact, shallow burrowers. During the recent work at the hydrothermal vents and the cold seeps vesicomyids have been found at Explorers Ridge, Juan de Fuca, Gorda Ridge, Santa Barbara Channel, Guaymas Basin, 21ºN, 13ºN, Galapagos Rift and 17º-22ºS in the Eastern Pacific. In the Gulf of Mexico they have been found at the Florida Escarpment and at the Louisiana Slope sites. To date, only species in the genus *Calyptogena* have been found at these sites except at the Louisiana Slope where both *Calyptogena ponderosa* Boss and *Vesicomya cordata* Boss occur (Fig. 2). The *Calyptogena* received from Dr. Kulm, Oregon State University, was taken from soft sediments in a subduction zone off the coast of Oregon (ALVIN dive 1428, 44º 56'N; 125º 21'W in 2461m). On the basis of the limited material available, it appears close to *C. magnifica* in size and general shell characters. If indeed it is this species then *C. magnifica* is the only known member of the Vesicomyidae to be both an infaunal burrower and an epifaunal nestler. Additional *in situ* observations and material are needed to prove this. Vesicomyids may well be good indicator organisms of sulfide/hydrocarbon environments and as such may also be of interest to paleoecologists. Hydrothermal vents can be readily detected by geologists, marine chemists and oceanographers by locating thermal anomalies, but a study of museum collections may well lead to the location of additional cold seeps or other soft sediment sulfide sites.

Thus, to date we know that at least one species of the typically infaunal eulamellibranch vesicomyids *(Calyptogena magnifica)* has become epifaunal and is found nestling in crevices of the lava rock and among the mussels at the hydrothermal vents. No lucinaceans (Lucinidae and Thyasiridae) have been found to do this. These families are typically shallow to deep-sea, infaunal eulamellibranchs found in areas where the oxygen tension of the water is low and the sediments are rich in sulfides. Thysirids are small, shallow to deep-sea infaunal sand/mud burrowers with thin shells that have subequal muscle scars and a weak hinge. Their gills, composed of large inner and small outer demibranchs or of inner demibranchs only, are thick due to the 'subfilimentar tissue which contains pigment granules and give the gills a brown color (Allen 1958). Lucinids are closely related to the thyasirids, but differ in having a sub-circular posterior adductor muscle scar and an elongate anterior adductor scar which turns inward from the pallial line. The gills have only a single pair of inner demibranchs which are larger and much thicker than those of *Thyasira* (Allen 1958; Kauffman 1969; Cavanaugh 1983a; Felbeck, *et al* 1983; Berg and Alatalo, 1984).It is now generally considered that lucinids have symbiotic, chemoautotrophic bacteria in their large fleshy brownish gills.

Stanley (1970) studied the burrowing habits of seven species of lucinids representing six genera and showed that all these shallow water species burrowed in organically rich muds, often among the roots of sea-grass in areas of low diversity. Using X-ray techniques he showed that most species lived with the ventral or posterior-ventral margin of the valves down, and, using their long, highly specialized foot (Allen, 1958, fig. 1; Kaufmann, 1969, fig. 91)), construct a mucus lined incurrent tube which extends from the anterior margin of the valves almost vertically to the surface. The excurrent tube, probably produced by the excurrent stream, may or may not be vertical and may not reach to the surface. From Stanley's X-rays it is apparent that these species produce from one to several small tubes extending from the ventral edge of the shell and radiating downward (Fig. 3). One can hypothesize that, while obtaining fresh clean water for respiration from the surface via the incurrent tube, the clam is extending its foot down into the sulfide rich muds and thus maintaining a supply of sulfide rich water for the symbiotic bacteria in the gills. Certainly carefully planned field and laboratory experiments are needed to prove this theory.

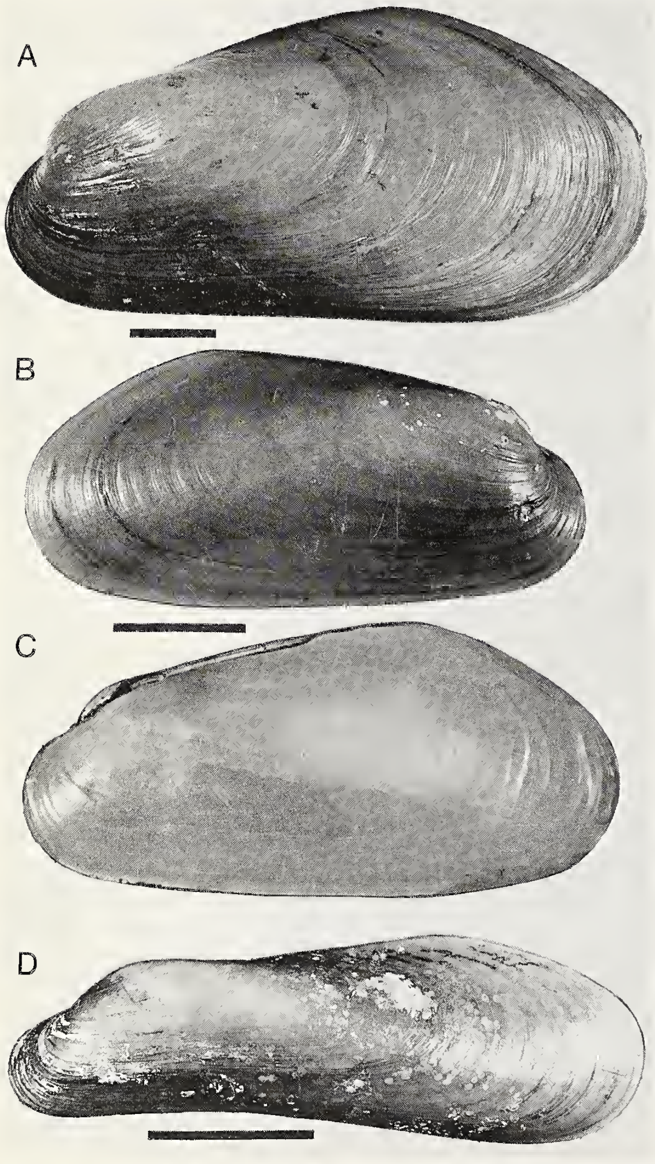

Fig. 4. Mytilids from the Galapagos and Florida. A. Galapagos Rift mussel from ALVIN Dive 983 at Rose Garden in 2600 m. Outer view of left valve (Scale bar = 10 mm). B-C. Young mussel from the Florida Escarpment (26° 02'N; 84° 55'W at 3266 m). ALVIN Dive 1344. B. Outer view of right valve. C. Inner view of same valve as in B. Note the similarity of the Florida specimen at this stage of development with the Galapagos species. (Scale bar = 10 mm). D. Adult mussel from the Florida Escarpment showing the great elongation of the shell with growth. (Scale bar = 50 mm). (Photos by R. D. Turner.)

Species of *Thyasira* have been found at the Guaymas Basin site and in pockets of soft sediment at 21°N in the Eastern Pacific (Grassle *et al.*, in press) and two species of *Lucinoma* (Family Lucinidae) were trawled at the Louisiana Slope site in the Gulf of Mexico (Fig. 2). One might expect to find lucinaceans at the hydrothermal vents but none have been found. Probably they are constrained to live in soft sediments because of their deep burrowing habit and their specialized method of anterior-posterior water circulation in the mantle cavity. As work at the hydrothermal vents and cold seeps progresses, additional species will no doubt be found at new soft sediment sites and in other pockets of soft sediments near the hydrothermal vents.

In the family Mytilidae only a few species are known to harbor symbiotic bacteria, possibly five out of an estimated 250 species for the family (Boss, 1971). The family is world-wide in distribution and ranges from the intertidal to abyssal depths (Allen, 1983). Most of the deep-sea species are small and have typical filibranch gills. Among these are *Dacrydium* (10 species, Allen, 1979), *Idasola, Adipicola, Modiolus, Crenella, Myrina, Amygadalum* and *Musculus* (Knudsen, 1970, 1979; Clark, 1962). All mytilids taken from the sulfide environments examined to date have large, fleshy, filibranch gills that harbor symbiotic bacteria which may be chemosynthetic but the fact that they are chemosynthetic and capable of CO_2 fixation has been demonstrated for the Galapagos Rift species only (Cavanaugh, 1983b; Felbeck *et al.*, 1983). The vent mytilids, like the vesicomyids, have a short gut (Kenk and Wilson, in press). The mantle cavity may be open as is typical of most mytilids or closed as in the Galapagos Rift species. Mytilids have been found at Explorers Ridge, 13°N, Galapagos Rift, Guaymas Basin and at 17°-22°S in the Eastern Pacific and in the Gulf of Mexico at the Florida Escarpment and the Louisiana Slope sites. The best known of the species collected to date is the one from the Galapagos Rift. It is large (Fig. 4) and abundant and so has been the basis of numerous anatomical, ecological, physiological and growth studies (Lutz *et al.*, 1980; Felbeck *et al.*, 1983; Cavanaugh, 1983a,b; Turner and Lutz, 1984; Le Pennec *et al.*, 1984; Smith, 1985; Kenk and Wilson, in press).

Based on the very limited material available for study, mussels found at the Guaymas Basin appear to be close to *Idasola*, a genus of small (average length 3 mm) wood associated deep-sea mytilids. This is based on shell characters only and though these are the smallest of the mytilids found at any of the sulfide/hydrocarbon sites, they are larger than any known *Idasola*. As Guaymas Basin is a soft sediment hydrothermal site (Grassle, 1983, 1984) located in an area where wood might well accumulate, it is possible that this species arose from a known *Idasola* (Fig. 5). Broader survey collections at the site and elsewhere in the Gulf of California are needed to prove this hypothesis.

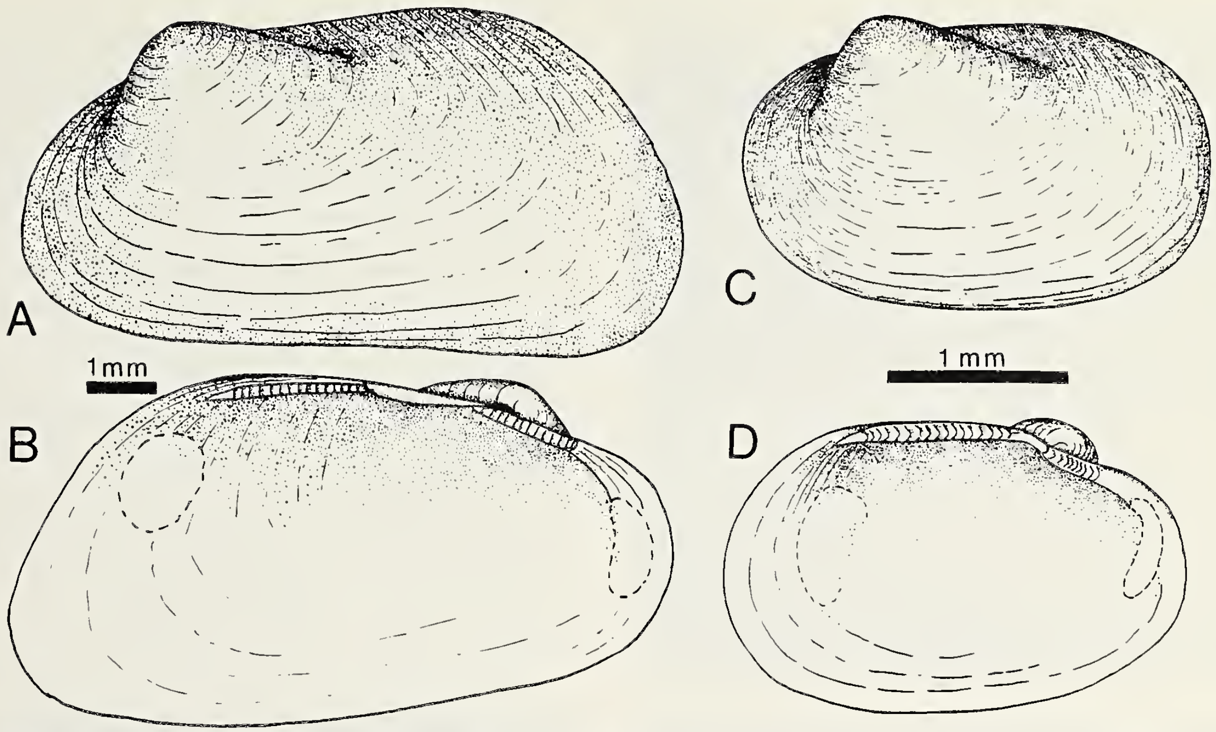

Fig. 5. Mytilid from the Guaymas Basin as compared with *Idasola*. A-B, Guaymas Basin mussel. A. Outer view of left valve. B. Inner view of left valve. C-B *Idasola argentea* (Jeffreys) from 'wood island' set at DOS-2 (38° 18.4'N; 19° 35.6'W in 3506 m). C. Outer view of left valve. D. Inner view of left valve. Note similarities in general shape, hinge structure and muscle scars between these two species. (Drawings made with the aid of a camera lucida by R. D. Turner.)

At the Florida Escarpment (26°2'N, 84°55'W in 3266 meters) the soft sediments are rich in sulfide minerals and hypersaline waters seep out from the base of the escarpment onto the seafloor (Paull, Hecker, Commeau *et al.*, 1984; Paull, Hecker, Neumann *et al.*, 1984). The most conspicuous members of the fauna are the tube worms *(Lamellibrachia,)* and the large, elongate mussels which may reach 7½ inches (19 cm) in length. The dense beds of mussels cover the dark sulfide rich sediments and their newly settled young commonly nestle in the eroded apices of the small coiled archaeogastropods which graze on the microorganisms living on the surface of the adult mytilids. These mytilids, like those of the Galapagos Rift, have large fleshy gills (Fig. 6) which harbor symbiotic bacteria (Cavanaugh, per. com). They differ from the Galapagos species by having an open mantle cavity and much more elongate valves as adults (Fig. 4D). Two species of mytilids have been trawled at the Louisiana Slope sites (27°45'N; 91°14'W; and 29°40'N; 91°32'W). One is a large (84mm long x 41mm wide), brown species with an open mantle cavity, and thick, fleshy gills typical of species harboring symbiotic bacteria. The other (*Amygdalum politum* Verrill and Smith) is a small, white, thin shelled species with typical filibranch gills.

On the basis of the very limited material at hand it appears possible that the mytilids at the sulfide/hydrocarbon sites arose from local species which became adapted to the sulfide environment and by harboring symbiotic chemosynthetic bacteria in their gills benefitted from the abundant nutrient source and were able to grow large. Where sufficient specimens are available to show growth series (Galapagos Rift [hard substratum], and Florida Escarpment [soft sediment] it would appear that these species grow as large and as fast as their intertidal relatives. Lutz has documented these observations with *in situ* experiments (Turner and Lutz, 1984).

Though there are a number of records of deep-sea Solemyidae (Dall, 1895, 1908; Clarke, 1962; Knudsen, 1970; Vokes, 1955, 1970) there are only two records of specimens from known deep-sea vent/seep areas. A single pair of empty attached valves of *Solemya (Acharax) caribbaea* Vokes was trawled at the Louisiana Slope and as they were still in fair condition it is probable living specimens were in the area. A single living specimen (Fig. 7) was obtained along with tube worms and a *Calyptogena* on ALVIN dive 1429 (44°56'N; 125°21'W in 2461 m) from soft sediments in a subduction zone off the coast of Oregon and was received from Drs. E. Suess and L. Kulm, College of Oceanography, Oregon State University. They state that "many solitary specimens, both dead and alive were seen lying on the surface or protruding from the sediment" (E. Suess *et al.*, in press). No doubt the specimens lying on the surface were all dead and the ones protruding from the sediment were probably repairing the incurrent or excurrent end of the upper U-shaped portion of the burrow - see following discussion of *Solemya* burrows and Figure 3. The calcareous portion of the single solemyid shell measures 13 cm in length, which is

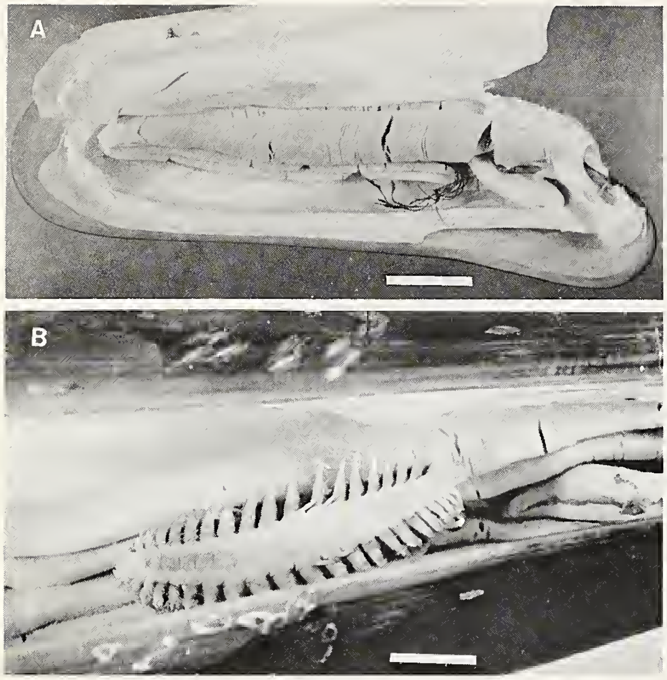

Fig. 6. Gills of the Florida mytilid. A. Right lateral view of the mussel with the right valve removed and the right mantle lobe reflected to show the long, thick gills and the elongate foot (Scale bar = 2 cm). B. Ventral view of gaping valves to show the thick gills and the large commensal polynoid polychaete which lives in the mantle cavity. Note the long narrow foot with its large byssal groove protruding from between the gills to the right of the worm. (Scale bar = 1 cm). (Photos by R. D. Turner.)

comparable with the 11.5 cm given by Dall for the holotype of *Solemya (Acharax) johnsoni* Dall 1891, from off Lower California (Albatross, station 3010, 27°23.45'N; 111°25.00'W in 1838 m). In 1895 Dall gave the range of *S. johnsoni* as from off Ecuador in 1740 fms [3182 m] to the Straits of Juan de Fuca. The Oregon specimen is probably this species but further material is needed to prove it. We obtained empty attached valves of another large species, *Solemya agassizi* Dall (calcareous portion of valves 6.4 cm in length) from the Panama Basin lying on the surface (ALVIN dive 1237, 05°20.65'N; 81°56.19'W in 3910 m). Attempts to obtain living specimens by dredging from the RV/Atlantis II, using a dredge with curved blades designed to bite deep into the sediment, produced only a fragment of what was a living specimen. This suggests that *S. agassizi* is a very deep burrower. The number of open, paired valves observed on the surface and the fragment dredged suggest that *S. agassizi* are living in fair numbers deep in the anoxic muds of the Panama Basin. Knudsen (1970) considered *S. agassizi* Dall a synonym of *S. johnsoni* and he is probably right but a comparison of our specimen from Panama Basin and a cotype of *S. agassizi* in the Museum of Comparative Zoology collection with the large specimen from off Oregon (Fig. 7) shows some differences. Obviously good series of well preserved specimens from several localities are needed to solve this systematic problem. The shell of the large Indo-Pacific species, *Solemya (Acharax) bartschi* Dall 1908, dredged between Ticas and Masbate Islands, Philippine Islands (Albatross, station 5215; 12°31.3'N; 123°35.24'E in 604 fms [1104m], measured 19.1 cm (calcareous portion) in length. Additional material and a careful study of these large species is essential before finally identifying the Oregon specimen.

It is well known that many solemyids have small guts (Stempell, 1899; Owen, 1961) and recently at least one species has been shown to be gutless (Reid and Bernard, 1980; Reid, 1980). There has long been a question as to how solemyids, particularly the large ones, were able to obtain sufficient nutrients with so small a gut, and various ideas were proposed to explain this (Stempell, 1899; Owen, 1961; Reid, 1980). Felbeck, Childress and Somero (1981) found sulfide-oxidizing enzymes in the gills of *Solemya panamensis* and suggested that this might help solve the mystery of *Solemya* nutrition, but it was Cavanaugh (1983) who presented microscopic (TEM), enzymatic, and physiological evidence of chemosynthetic bacteria in the gills of *Solemya velum* Say. It

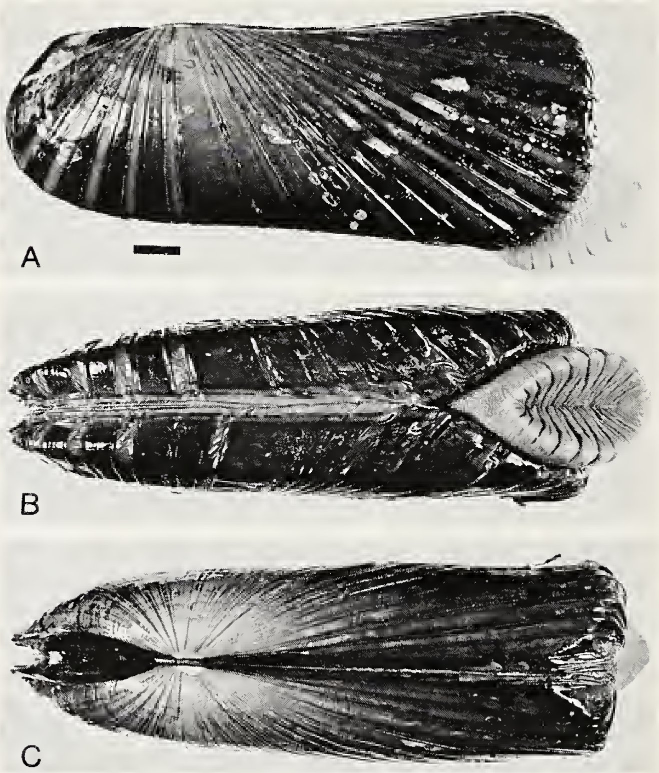

Fig. 7. The Oregon Solemyid from ALVIN Dive 1429 (44° 56'N; 125° 21'W) off the coast of Oregon in 2461 m. A. View of the right side of shell showing pronounced radiating sculpture and the foot protruding anteriorly. B. Ventral view showing the infolding of the 'velum' (uncalcified portion of the valves) when the animal is contracted. Note folded foot. C. Dorsal view showing the posterior position of the umbos, the radiating sculpture and the tip of the foot protruding anteriorly (Scale bar = 10 mm). (Photos by R. D. Turner.)

now remains to isolate and characterize these symbiotic bacteria and to determine their role in the nutrition of their hosts. Several authors have referred to the mucus lined burrows of solemyids, while Frey (1967) illustrated an *in situ* Y-shaped burrow and Stanley (1970) using X-ray techniques illustrated Y-shaped burrows made by *S. velum* in a flat aquarium in the laboratory. Stanley stated that the animal usually rests in the lowest portion of the U-shaped part of the burrow, i.e. above the vertical portion of the burrow which extends down into the sulfide rich muds. As with the lucinaceans (Lucinidae and Thyasiridae), the solemyids have an anterior-posterior water current through the mantle cavity so that, while obtaining oxygenated water for respiration through the incurrent tube, they can obtain sulfide rich water for the symbiotic chemoautotrophic bacteria in the gill through the ventral aperture from the tube below. It would appear that in the solemyids and the lucinaceans we have a rather remarkable case of parallel evolution. These two groups of bivalves, widely separated phylogenetically, i.e. Protobranchia [Solemyidae] and Heterodonta [Lucinidae and Thyasiridae], have evolved the same means of utilizing sulfide rich environments. In both of these groups the gills have become enlarged to accommodate the symbiotic bacteria, the food groove and labial palps are poorly developed, the respiratory water current is well developed and anterior-posterior, the digestive tract is short and simplified and, interestingly, they both live at the bottom of more-or-less U-shaped burrows from which they extend tubes down into the sulfide muds below (Fig. 3). As mentioned earlier for the lucinids, the solemyids also appear to be constrained to live in soft substrata because of their deep burrowing habit and unusual method of obtaining sulfides for the symbiotic chemosynthetic bacteria. Though we know that *S. velum* can be kept alive for a week or more lying in a dish of aerated sea water when reduced sulphur compounds are added (Cavanaugh, 1985), we know of no surface living species and it is unlikely that, with their delicate shells, they could survive nestled among rocks or mussels at a thermal vent site.

The gastropods are less well known than the bivalves and, except for the archaeogastropods (both patelliform and coiled), are generally rare and/or inconspicuous. They have not been used in experimental work and, other than the limpets, are only casually mentioned by physiologists and ecologists except by those interested in life histories, larval dispersal and the means by which these 'sulfide islands' are populated (Turner *et al.*, in press). Mollusks, particularly gastropods, then become important, for they lend themselves to such a study. They are the only taxa (at the vents and seeps) for which the early ontogeny is recorded in the shells of the juveniles and well preserved adults (Lutz, Jablonski *et al.*, 1980, 1984; Turner and Lutz, 1984). Perhaps the major reason for the apparent 'neglect' of the gastropods is that they are far more difficult to identify than bivalves. Knowledge of the anatomy, particularly the radula, is often needed for the postive identification of a species and is essential for the description of new genera or species. Except for the archaeogastropods, the number of specimens available per species is usually very limited and often only the shells are known. In some cases, it may take years to accumulate sufficient material to allow for the description of a new species. Sieving the water remaining in the bottom of ALVIN retrieval buckets and the washings from all specimens and equipment brought to the surface has proved the best means of obtaining small gastropods. The limpets live on the vestimentiferan tubes and mussels and are brought up in great numbers with these large specimens.

There are far more species of gastropods (subclass Prosobranchia) than bivalves at the vents and seeps (see pictorial map, Turner and Lutz, 1984), but unfortunately most cannot be placed readily into families and comparisons are usually made at the level of the Order (i.e. Archaeogastropods, Mesogastropoda, Neogastropods). The archaeogastropods are the most numerous both in number of species and of individuals, particularly at the hydrothermal vents (Turner and Lutz, 1984). The archaeogastropod limpets are being studied by Dr. James McLean, Los Angeles County Museum of Natural History and Dr. Vera Fretter, Zoology Department, University of Reading. *Neomphalus fretterae* McLean, the largest of the vent limpets, was described in 1981 and, in a companion paper, Fretter, Graham and McLean (1981) detailed the anatomy of this interesting filter feeding limpet. In addition to the superfamily Neomphalace McLean 1981 and family Neomphalidae McLean 1981, three new families each differing from the others and the Trochacea at the superfamily level have been obtained from widely separated hydrothermal vents. Fretter and McLean are collaborating on the descriptions of these three families (McLean, 1984). In a letter dated May, 1985 Dr. McLean writes that "there are now known to be a total of 23 limpet species in five different superfamilies but only one of these from a cold seep, the Florida Escarpment site." Since that writing a single limpet was trawled from the Louisiana Slope site. In addition to the limpets there are a number of coiled archaeogastropods at vents and seeps, usually with two to four species at the vents and at least one at each of the seep areas; three species of Trochidae have been collected at the Louisiana Slope site. Typically there are proportionately fewer species of mesogastropods in the deep-sea and this also appears to be true at the vents. These are largely being studied by Anders Warén, Zoologiska Institutionen, Göteborg, Sweden and Phillippe Bouchet, Museum National d'Histoire Naturelle, Paris. Of the relatively few neogastropods collected, most are small and belong to the family Turridae.

In Figure 8 Dr. Michael Rex (University of Massachusetts, Boston) compares the taxonomic composition of prosobranch snails collected from the soft-bottom, deep-sea environment of the western North Atlantic to that of three hydrothermal vents in the eastern Pacific and a sulfide seep off Florida. The three curves, representing

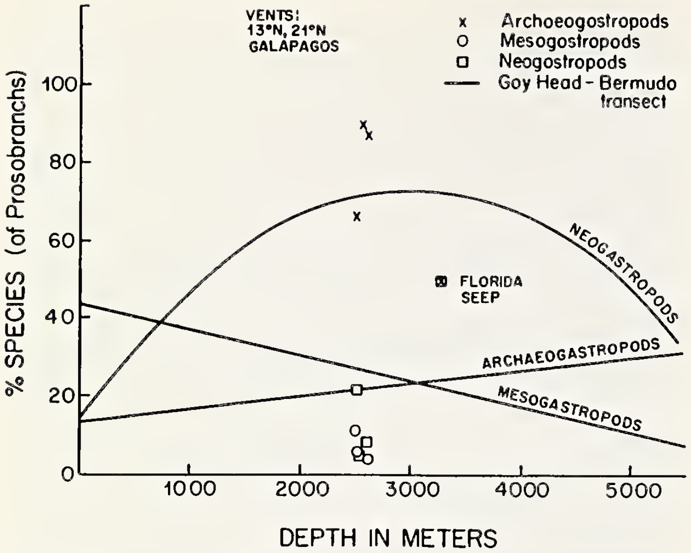

Fig. 8. A comparison of taxonomic composition of prosobranch snails from the deep sea of the western North Atlantic with three hydrothermal vents (Galapagos, 13^0N, 21^0N) and the Florida sulfide seep. The three curves are fitted regressions (equations below) of the proportions of the three prosobranch orders with depth in the western North Atlantic (data from Rex and Warén 1982, with the addition of *Lacuna cossmanni*, see p. 172). Proportions of the three orders from vents and the Florida seep are from Turner and Lutz (1984). Regression equations (N=20) are:

ARCHAEOGASTROPODS: $Y=13.56530+.003385X$, $r=.51$, $P<.05$

MESOGASTROPODS: $Y=43.77800-.006554X$ $r=.84$, $P<.01$

NEOGASTROPODS: $Y=13.36260+.039897X-.000007X^2$, $r=.74$, $P<.01$

(Graph by M. Rex)

the western North Atlantic fauna, are fitted regressions of the proportion of prosobranch orders against depth. The curves, while statistically significant (see figure caption), are meant only to show the general taxonomic trends with depth. The analysis is based on data published by Rex and Warén (1982) comprising 16,733 individuals distributed among 108 species from 20 epibenthic sled samples collected along the Gay Head-Bermuda transect (478-4970m). The proportions of archaeogastropods and mesogastropods tend to increase and decrease, respectively, with depth in the western North Atlantic. Neogastropods, which are more diverse throughout most of the bathymetric range, show a parabaric pattern, dominating the assemblage at mid-bathyal depths and being less well represented at upper slope and abyssal depths.

The relative proportions of the three orders from the hydrothermal vents at 13^0N, 21^0N and the Galapagos (Turner and Lutz, 1984) are plotted in Figure 8 at the approximate depth where vents occur (2600m). Vent faunas have a much higher proportion of archaeogastropod species and a much lower proportion of neogastropods than found in the western North Atlantic. When the combined vent communities are compared to the continental rise fauna (2000-4000m) of the western North Atlantic, the representation of the archaeogastropods is found to be significantly higher at the vents. ($X^2=34.33$, d.f.=1, $P < .001$, two-tailed test on raw data of species numbers; Siegel, 1956).

Among archaeogastropods, there is a higher incidence of limpets at the vent sites. In the entire western North Atlantic collection there are only 5 limpet species, and never more than 2 species per sample (median = 1 species). In contrast, collections from the three vents yielded 6-15 limpets, which make up 60-67 percent of prosobranch species recovered. When vent communities are compared to the rise fauna of the western North Atlantic, vents are revealed to support significantly more limpets (P=.033, two-tailed Fisher-Yates exact probability using the tables of Finney *et al.*, 1963).

The snail faunas of sulfide seeps are more poorly known than those of vents. Figure 8 shows the taxonomic composition of the Florida seep (Turner and Lutz, 1984), which is presently the best known cold seep site. It appears to support a fauna that differs taxonomically from those at comparable depths in the western North Atlantic. But with only two archaeogastropods and two neogastropods collected at the seep so far, it cannot be compared to other situations with any statistical confidence.

The difference in per cent species of archaeogastropods at the vents and at a deep-sea soft-bottom area is striking. The obvious answer is the rocky substratum of the vents, but, when compared with a rocky littoral zone or a deep-sea 'wood island' set 304 km south of Woods Hole in 3500m or in the Tongue of the Ocean, Bahamas in 2600m the ratios remain essentially unchanged. A possible explanation may be in the reduction of predators at the vents. As noted earlier, the toxic sulfides at the vents are in the water column while at the seeps (where limpets are neither abundant nor diverse), they are in the muds and the water column is essentially unaffected. All organisms living at the hydrothermal vents must adapt to these toxic substances in the water column whereas at the seeps apparently only those species living in or in close contact with the sulfide rich muds are affected. Cohen and Haedrich (1983) when reporting on the fish of the Galapagos vents noted 1) that the 'species diversity and population density of fishes overall decrease closer to the vents. Opposite trends appear to characterize invertebrates'. 2) that the 'number of individual fish that congregated around the bait camera in the Galapagos was surprisingly low' and 3) that 'predation by fishes within the vents, where species like the large and gaping vesicomyid clams and vestimentiferans ought to be especially vulnerable, must be relatively unimportant'. As noted by Connell (1971) predators are far more affected by adverse stress conditions than their prey. Apparently no predatory fish have become adapted to live in the noxious water and prey on vent organisms, perhaps because of the transient nature of the vents, but that seems highly unlikely because fish could easily swim between vents. According to Dr. Bidigare, Department of Oceanography, Texas A. and M. University (personal communication), there was no lack of predatory fish around the Louisiana Slope site, and at the Oregon site they were "impressed by the speed with which carnivorous fish and a crab were attracted to the site" and "by their ferocious feeding on specimens of giant clams crushed by ALVIN." (Suess *et al.*, in press). Interestingly at both these sites the water temperature is ambient and the toxic elements are largely in the sediments rather than the water column as at the vents.

Stanley in 1982 presented a new and interesting theory relating the origin of gastropod torsion to predation and the need for an operculum. He argues that it was virtually impossible for coiled but untorted monoplacophorans to evolve an operculum and suggested that the post-Cambrian decline of that group paralleled the evolution of predators such as crabs, carnivorous snails and bony fish. Stanley also noted that observations on a living species (*Vema* sp.) by Lowenstam (1978) indicated that monoplacophorans could not clamp onto the substratum like modern limpets (i.e. Acmaeidae, Patellidae) and so were vulnerable to predators. This would suggest that vent limpets, like intertidal species, can clamp down tightly and so protect themselves from predators. We know very little about the behavior of the vent limpets but two observations suggest that they may not clamp down as strongly as littoral species. First, observations made from ALVIN, the study of films, tapes and still pictures suggest that at least some species of vent limpets seem to move about slowly with their shells raised well off the bottom. At such times they appear very vulnerable to predation. Secondly, unlike limpets on the 'wood islands' which usually remain attached during retrieval, limpets at the vents usually have fallen off the sustratum by the time they reach the surface. If vent limpets are more like monoplacophorans in not clamping tightly to the substratum and if the limpets at the seeps are related to those at the vents as suggested by McLean (personal communication, B. Hecker) for the species at the Florida Escarpment, then it is easy to understand why there are so few limpets at the seeps where predators may be prevalent. One can theorize that the noxious vent waters protected the limpets against predation by fish and the abundant food supply allowed them to become extremely abundant and diverse. The dominant archaeogastropod at the Florida seep is coiled and operculate and may support Stanley's theory on 'predation and the opercular imperative'.

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MOLLUSCAN MUCINS: A UNIFYING THREAD

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ABSTRACT

Mucocytes and the ability to produce mucins are ancient traits of many phyla including the Mollusca. While mucus secreting structures are primitive, variations of these glands reveal apomorphs that offer clues to the phylogeny of many molluscan groups. The diverse functions that mucins play in molluscs are ramifications of a basic functional pattern that initially involved locomotory lubrication, burrow support, cleaning, filter feeding, and other "basic" molluscan activities. Ancestral pedal mucocytes, for instance, are an example of the stock "anlage" for a variety of extant molluscan activities including larval cements, byssal production, suspension threads, homing, and feeding lines. Arenophilic mantle glands of anomalodesmatan bivalves constitute an example where epithelial mucous glands offer support to proposed phylogenies. Recent molluscs have mucocytes and mucous glands that might be "preadapted" for their present functions. Early mucoid sheaths could have enveloped the dorsum of ancestral molluscs and could have been "preadapted", for instance, for absorption or adsorption of calcium and carbonate ions leading to an incipient calcareous shell. These examples and trends offer new insights into molluscan phylogeny and reveal potentially valuable characters for future research into the evolution and taxonomy of the phylum.

> "We are far too ignorant, in almost every case, to assert that any part or organ is so unimportant for the welfare of a species, that modifications in its structure could not have been slowly accumulated by means of natural selection."
> C. Darwin (1859)

> "Zoologists are not new to studies on mucus . . ."
> S. Jakowska (1965)

> "Molluscs' reputation for being "slimy" is easily attested to . . . "
> M. Denny (1983)

In 1963, in concluding remarks at a Symposium on Mucous Secretions, W. Pigman opined that the era of neglect in studies of mucus had come to an end. The resounding truth of that statement is evidenced in the plethora of publications that have appeared since the mid 1960's dealing with the chemistry, form, and function of mucoid substances. Malacologists, in particular, have long appreciated the functional merits of the variety of mucins produced by members of the Mollusca. While well appreciated, the subject of these secretions is little understood, and the era of study of molluscan mucins is far from over.

The multitude of functions mucins play within molluscs include roles in protection, locomotion, respiration, reproduction, ingestion, egestion, predation, osmoregulation, cleansing, luminescence, geographic dispersal, aestivation, homing, shell production, byssal attachment, etc. In fact, it is difficult to think of many molluscan activities that do not involve mucins. Several of these diverse and important functions will be discussed and common features of each will be examined. With this background as a basis, I will attempt to demonstrate the unifying aspect of mucins in the evolutionary development of the Mollusca.

Secretions are not usually considered a unifying evolutionary characteristic. Character states typically take the form of various morphological, biochemical, behavioral, or physiological traits. We typically overlook the fact that the product of these traits is a secretion, and *that* secretion is often mucus.

The *initial* mucoid secretion developed over 3,000 million years ago with the rise of early bacteria and production of bacterial mucilagenous coats. The "slime" layers probably functioned very early in the protection of procaryotes. These simple capsular coats were early examples of the importance of mucus in all organisms, then and now. The evolutionary history of mucoid

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products is indeed a fascinating one, but upon which we can only speculate. Nevertheless, the rich literature on molluscan mucins allows some conjecture on the subject within the Mollusca.

There is an intriguing yin-yang embodiment within many molluscan mucous secretions. They are used, for example, to compact both food for ingestion and wastes for egestion in bivalves, as predation and antipredation devices in some naticid gastropods, in pedal lubrication to ease locomotion as well as to secure adhesion in many terrestrial pulmonates, during burrowing and flotation transport in the bivalve *Corbicula* c.f. *fluminea* (Müller), and in shell formation and shell dissolution by many muricid gastropods. This functional polarity is common within the Mollusca and represents a unique viscoelastic property of mucins. Subtle differences exist within the mucins that allow these divergent activities. The basic chemistry of mucus is rather conservative, but differences, subtle though they be, are the foundation for the broad array of uses to which molluscs put mucins.

GENERAL MUCIN CHEMISTRY

A wide array of terms has "evolved" with our knowledge of mucins. While Denny (1979) has stated that "The term 'mucus' has never been precisely defined", excellent reviews of the basic chemical composition of mucins, their diversity and uniformity, can be found in Hunt (1970) and Denny (1979, 1983). Briefly, mucus, or mucins, are secretory products divisible into two large categories. Both entail a complex of polysaccharide and protein components, the primary ingredient always being water [up to 99.7% water in the hypobranchial gland mucus of *Busycon canaliculatum* (Linné) (Hunt, 1970)]. Superficially "all molluscan mucins are very much alike" (Denny, 1983). Mucopolysaccharides are mucins dominated by a polysaccharide component, while glycoproteins have a dominant protein portion. The latter are essentially proteins with less with 4% hexosamine (carbohydrate portion). Usually the protein and carbohydrate portions of a glycoprotein are covalently bound but this is not true for mucopolysaccharides (Hunt, 1970). Glycoproteins can have one or many heterosaccharide units bound along the protein chain (Hunt, 1970). Typically, neutral sugars are found in glycoproteins and not mucopolysaccharides (Denny, 1983). All mucopolysaccharides are large anions that readily attract cations such as Na^+ and K^+. There is nothing truly unusual about these protein-polysaccharide complexes and essentially molluscan mucins are quite similar to most other mucins.

There are, of course, important differences in the specific chemical composition of the various mucopolysaccharide and glycoprotein complexes. Differences in amino, neutral, and acidic sugars in the polysaccharide components, and distribution of amino acids in the protein components, all lend distinction to various mucins. The solid component of mucus (i.e., proteins, polysaccharides, and a small amount of inorganic salt) usually accounts for much less than 10% but this small amount of material is responsible for the qualities of the mucin itself. For example, acid polysaccharides are large polyvalent anions that very tightly bind cations. Sodium and potassium cations, in fact, can be so effectively bound that they appear non-ionic (White *et al.*, 1973). These large anions have a tendency to aggregate, enhanced by polyvalent cations such as Ca^{++}.

While mucins in molluscs can generally be thought of as having subtle differences, they are basically typical mucopolysaccharides or glycoproteins with protein and carbohydrate portions being the "active" components. Denny (1983) listed some of the monosaccharide subunits composing the polysaccharide portion of some mucins. His list shows acidic monosaccharides dominating with some amino and neutral sugars occurring less prevalently. This generalization comes from a list of only six species of gastropods, a reflection of just how little we really know about these substances. Proteinaceous portions of mucins of molluscs also are known from an equally small group of species of snails, and show that serine and threonine are often present in high proportions. Cysteine is typically present, and there are a higher number of acidic than basic amino acids.

Hunt (1970) outlined a suggested evolution of mucopolysaccharides and glycoproteins. Bases for much of his speculation are interphyletic similarities of biochemical pathways involved in the biosynthesis of various polysaccharides. This ubiquitousness argues for synplesiomorphy. Not only are biochemical pathways similar, but the cellular mechanisms involved in synthesis of mucins in eucaryotes are also similar across the phyla (see, for example, Storch and Welsch, 1972).

MOLLUSCAN MUCIN FUNCTIONS

There is no single source available that summarizes in detail the functions of molluscan mucins (see Table 1). Here I will limit discussion to a few functional examples from the many available and show that certain generalizations and assumptions can be made concerning molluscan mucins. First, mucins are an intricate and basic part of the life of every mollusc. No detailed works have been published on the functional morphology of molluscs that do not mention mucocytes or mucous secretions. Secondly, strong comparative evidence demonstrates that the basic chemical structures of mucins are similar. This similarity resides in the glycoprotein or mucopolysaccharide backbone of these secretions. Thirdly, the structural and chemical similarities in molluscan mucins signify that mucocytes of molluscan "stem-groups" were quite simple and plastic. This assumption would allow for great evolutionary diversification in subcomponents, forms, and functions. And fourthly, discrete apomorphs can be selected from among the trends of parallelism that otherwise would leave the phylogenist distraught. Specific mucus secreting organs can be so solitary in their occurrence as to leave little

Table 1. Molluscan mucins: a partial list of functions, sources and references.

FUNCTION	MOLLUSCAN SOURCE(S)	REFERENCES
locomotion (pedal lubricant)	patellid and acmaeid limpets	Grenon and Walker, 1978
	acochlidiaceans	Robinson and Morse, 1979
	pulmonates	Jones, 1973, 1975
	Corbicula fluminea (juveniles)	Kraemer, 1977
trailing and homing	*Ilyanassa obsolita*	Trott and Dimock, 1978; Bretz and Dimock, 1983
	Acmaeia scabra	Hewatt, 1940
	Siphonaria alternata	Cook and Cook, 1975
	Littorina irrorata	Hall, 1972
adhesion to substratum	*Patella vulgata*	Grenon and Walker, 1978
	Ostrea edulis (larva)	Cranfield, 1973
	Pecten maximus (larva)	Gruffydd *et al.*, 1975
adhesion of sediment to shell	lyonsiids	Prezant, 1979a, 1981a
	verticordiids	Allen and Turner, 1974
byssal attachment	*Chlamys islandica*	Gruffydd, 1978
	Mytilus edulis	Tamarin *et al.*, 1976
	Anomia simplex	Prezant, 1984
geographic dispersal	patellids	Vahl, 1984
	Corbicula fluminea	Prezant and Chalermwat, 1984
	Janthina janthina (Linné)	Morton, 1979
	hydrobiids	Newell, 1962
aestivation	pulmonates (epiphragm)	Machin, 1964
food lubricant, enzyme carrier	*Helix* spp.	Moreno, *et al.*, 1982
filtration	*Crepidula* spp.	Jørgensen *et al.*, 1984
prey drilling	muricids, naticids, cassids	Carriker, 1978, 1981
	(accessory boring organ,	Hughes and Hughes, 1981
	pedal glands)	Houbrick and Fretter, 1969
paralytic secretions	muricids	Whittaker and Michelson, 1954
	Octopus vulgaris	Mebs, 1973
"farming"	limpets	Connor and Quinn, 1984
pallial cleansing	*Busycon* spp., *Buccinum* spp.	Ronkin, 1952; Hunt and Jevons, 1963, 1966
	Mercenaria mercenaria	Hillman, 1964
antidessication	pulmonates	Machin, 1965
immune system	*Biomphalaria glabrata* (Say)	Bayne, 1983
	opisthobranchs	Thompson, 1960
	naticids	
luminescence	pholads	
osmoregulation	gastropods	Machin, 1974
ion transfer	*Mercenaria mercenaria*	Hillman, 1969
shell matrix	*Helix aspersa, Mytilus edulis, Mercenaria mercenaria*	Wilbur and Saleuddin, 1983; Wada and Fusahashi, 1971; Abolins-Krogis, 1963; Uozumi and Suzuki, 1979; Greenfield *et al.*, 1984
reproduction	Arionidae (courtship)	Quick, 1947, 1960
	Zonitidae (courtship)	Barr, 1928
	prosobranchs (spawn masses)	Fretter and Graham, 1962
	Ilyanassa obsoleta (Say) (egg capsule)	Sullivan and Maugel, 1984
	Corbicula fluminea (brood)	Morton, 1977
	Octopus vulgaris (egg adhesion)	Wood, 1963
debris binding and expulsion	*Dentalium* spp.	Stasek and McWilliams, 1973
feeding and respiratory tubes	*Thracia phaseolina*	Yonge and Thompson, 1976
	Cochlodesma praetenue	Allen, 1958

question as to their relatively recent derivation in certain taxa (when viewed in a total phylogenetic picture). While the "typical" mucocyte is ancient in origin, some of the highly complex and specialized mucous glands of some molluscs are probably more recently evolved and offer clues to the phylogeny of some molluscan taxa.

LOCOMOTION: VISCOELASTICITY OF MUCINS

Secretion of mucus is of critical importance to pulmonates during locomotion (Jones, 1973, 1978; Denny, 1979). This mucus requirement in gastropod locomotion has been well established, not only in pulmonates but also in all other "crawling" and "burrowing" gastropods examined. Parker (1911) suggested that two types of mucus were typically produced during gastropod locomotion, a low and a high viscosity form. The high viscosity mucus probably is produced by suprapedal glands, and the low viscosity mucus by the pedal sole. The latter mucus fills spaces caused by muscular waves running down the foot. Ciliary movement, normally beating backwards in these snails, likely functions in spreading the mucus evenly over the foot (Jones, 1973). The significance of pedal mucins in locomotory activity was exemplified in *Helix aspersa* Müller by Jones (1975). Tests showed that this land snail can drag 50 times its own weight along a horizontal surface and nine times its weight along a vertical incline, and that these activities were limited not by pedal musculature but by mucoid adhesiveness.

Grenon and Walker (1978) described the pedal glandular systems of two limpets and found them important in locomotion and adhesion (some pedal glands along the sides of the foot apparently are involved with antidessication). Lane (1984) described the pedal mucous glands of the pulmonate *Cionella lubrica* (Mueller) and found that its foot "conforms rather closely to a general molluscan model." He suggested that some pedal mucins of *C. lubrica* function in locomotory lubrication, respiration, and antidessication, functions probably common throughout the Class. Even in very small gastropods pedal mucins play an important role. The pedal glands of the acochlidiacean *Unela nahantensis* Doe secrete mucosubstances involved in locomotory lubrication and binding of sediment particles (Robinson and Morse, 1979).

The viscoelastic properties of mucins (i.e., dual functionality in response to shearing forces, acting to resist flow under one instance and flowing under another), allows these secretions to act effectively in punctuated locomotion. Thus, molluscs are usually able to maneuver effectively, albeit slowly, upon and within the substratum, negotiating sharp turns, stops, vertical inclines, waves, and inverted surfaces. However, the physical properties of mucins that allow these locomotory activities are not understood.

Nowhere is the viscoelastic property of mucus better exemplified than in the retrograde pedal waves of the limpet *Patella vulgata* Linné. In locomotion of this kind the pedal mucus must change from a solid to a liquid phase in response to pedal pressures (muscular or hemocoelic). Denny (1980) proposed a model whereby contraction of the dorsoventral musculature of the foot along the leading edge of pedal muscular waves (posteriad moving waves) stimulates the pedal mucus to change into a fluid stage. The fluid mucus is then "healed" into a solid stage along the trailing edge of each wave. Thus, the pedal sole moves beneath pedal waves and need not be "lifted" from the substratum. Denny (1980) has similarly shown that pedal mucins of the slug *Ariolimax columbianus* (Gould) change from liquid to solid (or gel) stage in a short period of time (0.15 sec), allowing the snail to use pedal waves to "ratchet" forward over a linear series of sol-gel phases (Denny, 1980; Trueman, 1983).

Gastropods and other molluscs with a dorsoventrally flattened foot are not the only members of this phylum to make use of pedal mucins for locomotion. Motile bivalves, especially juveniles and very small clams, are able to glide over the substratum using their extended foot in much the fashion of a snail (Kraemer, 1977). Lyonsiid bivalves (esp. *Lyonsia* spp.), Tellinaceans, Sphaeriaceans, several venerids, and many other bivalve molluscs are able to creep with their foot extended using mucociliary locomotion leaving a thin mucous trail behind (pers. observations).

The burrowing behavior of bivalves, scaphopods, and gastropods is well known (Trueman, 1983). However, little mention is made in the literature of the fact that the active foot, or propodium of the foot, of these molluscs, so effectively used during burrowing, is not only dependent upon muscular and hemocoelic pressures, but also upon its ability to overcome frictional forces between sediment and pedal epithelium. This is possible because of mucoid lubricants.

Representatives of the Aplacophora also produce pedal mucins. Hoffman (1949), for instance, reports that the pedal groove and pedal pit of some aplacophorans are packed with multicellular glands that are probably mucous glands. (It is common to regard the pedal fold or folds of "solenogasters" as corresponding to the foot of other molluscs.) Pruvot (1899) and Baba (1940) reported strands of mucus that were released from the pedal pit or groove of some aplacophorans. Functions of these mucoid threads are not well understood.

The foot of some polyplacophorans consists of alternating tall columnar cells and mucocytes (Plate, 1901) or anterior rows of mucocytes (Knorre, 1925). There are few recent data on chiton pedal glands, but presumably the glandular epithelium, dense with mucocytes, serves functions similar to those gastropods.

Lemche and Wingstrand (1959) described mucocytes in monoplacophoran epidermis. Very likely pedal mucins are involved in locomotory lubrication of these limpet-like forms.

Comparable pedal activities found in gastropods, polyplacophorans, scaphopods, bivalves, and monoplacophorans suggests parallel development of the foot-mucin-muscle complex. However, virtually no phylogenetic record exists to support common origin, parallelism, or even convergence within the pedal complex of molluscs. In the case of pedal mucins, based on similarities of structure and function, and the presumed distant common ancestry within "flat-worm" taxa (c.f. Stasek, 1972; Stasek and McWilliams, 1974; Salvini-Plawen, 1980), we can conjecture that pedal mucins have long been operative and are indeed a common and central plesiomorph stemming from a long extinct group of common ancestors. From this ancestral stock we can hypothesize a polyphyletic evolution involving loss, reduction, and modification that has resulted in the current diversity of pedal complexes.

ADHESION AND ATTACHMENT

The limpet *Patella vulgata* possesses nine different pedal glands that open onto some portion of the foot (Grenon and Walker, 1978). Some of these are certainly active in lubricating the foot during locomotion; others, however, are involved in adhesion. The former lubricatory secretions are primarily low viscosity acidic mucopolysaccharides. The adhesive secretions are highly viscous acidic mucopolysaccharides. The acochlidiacean, *Unela nahantensis*, has pedal glands involved in adhesion (Robinson and Morse, 1979) as does the larval foot of the oyster *Ostrea edulis* Linné and the scallop *Pecten maximus* (Linné) (Cranfield, 1973; Gruffydd *et al.*, 1975, respectively).

Attachment of byssal threads to firm substrata is a common activity among many mytilized and laterally compressed bivalves. Waite and Tanzer (1981) described a polyphenolic tanning system in *Mytilus edulis* Linné producing flexible, strong byssal threads. The byssus of anomiid bivalves has become calcified (Morton, 1979; Prezant, 1984). In each of these systems a mucoid substance is often the first secretion laid down by the foot prior to attachment of proteinaceous byssi. Gruffydd (1978) described pedal mucous glands along the sole of the foot in *Chlamys islandica* (Müller) that empty into the byssal groove. The first indication of byssal production in *C. islandica* is the preparation of the substratum by the bivalve, which sweeps its foot over the surface and lays down a layer of mucus. This somehow prepares the substratum for byssal thread attachment. In *Mytilus edulis* a mucosubstance is also secreted by the distalmost part of the foot and applied to the substratum prior to byssal attachment. The tip of the foot of *Anomia simplex* Orbigny is densely packed with mucocytes. (Prezant, 1984) and it is likely that the calcified byssus of this bivalve is also preceded by deposition of a mucoid film on the substratum.

A large number of histochemical and biochemical tests have shown, in at least *M. edulis*, that there are three primary secretions involved in byssal thread production; polyphenol, collagen and mucus (Brown, 1952; Pikkarainen *et al.*, 1968; Pujol *et al.*, 1970; Tamarin *et al.*, 1976). Mucoid products found in the byssal plaque of some mussels appear to have the proper molecular qualities to play at least an incipient role in primary adhesion of the plaque to the substratum (Tamarin *et al.*, 1976). Mucins might also act as localized regions of "tackiness" to stabilize plaque material in the process of polymerizing (Chen and Cyr, 1970). [Similar events take place in the polymerization of the outer egg capsule coat of some muricid snails (Tamarin and Carriker, 1968.)]

Waite and Tanzer (1981) suggested that wetting by mucins is a "crucial element" for byssal adhesion and acts to allow close contact between adhesive and substratum. In addition, Gruffydd (1978) found that mucins contribute "to the matrix which fills the spaces between (byssal) ribbons" in some scallops.

TRAILING BEHAVIOR: MUCINS AS A CARRIER

One of the important features of mucin is its capacity to act as a carrying matrix for active and otherwise ephemeral substances. This chemical relationship relegates specific features to various mucoid secretions. One such feature is illustrated by the intra- and interspecific trail following behavior of many gastropods. The marine prosobranch *Ilyanassa obsoleta* (Say) has been particularly well examined for this ability (Trott and Dimock, 1978; Bretz and Dimock, 1983). Polarity of these mucoid trails has been implicated in this behavior (Trott, 1978; Trott and Dimock, 1978; Dunn, 1982), but our knowledge of actual mechanisms involved is still vague. Cook (1971) suggested that if chemoreception is involved, as is thought by Trott (1978), Trott and Dimock (1978) (for some nassariads) and Hall (1972) and Peters (1964) (for some littorines), then there must be some persistent substance bound in the mucus to allow trails to remain active for as long as two days.

Mucous trails in gastropods are also selfserving in that they have been implicated in the homing behavior of *Acmaeia scabra* Gould, *Siphonaria alternata* Say, and *Littorina irrorata* Say (Hewatt, 1940; Cook and Cook, 1975; and Hall, 1972, respectively). Intraspecific trailing behavior in *I. obsoleta* near the onset of winter may lead to extensive aggregate migrations to subtidal positions (Jenner, 1956, 1957, 1959).

An interesting recent find concerns mucous trails of some intertidal limpets that act as adhesive traps for microalgae, the snail's principal food source (Conner and Quinn, 1984). In fact, for two limpets examined by Connor and Quinn (*Lottia gigantea* (Sowerby) and *Collisella scabra* Gould), mucoid trails not only trapped algae but stimulated algal growth. The significance of this discovery resides in the capacity of many limpets to "home" and retrace their trails, ingesting mucus and attached material (food) as they do. The expense of this behavior (9-26% of assimilated energy) (Calow, 1974; Denny, 1980c) is defrayed by the benefit. Calow (1979) also found that the mucus produced as a pedal trail by the pulmonate *Lymnaea peregra* (Müller) was prone to rapid bacterial degradation. He suggests it is advantageous for this snail to have a mucoid trail vulnerable to bacterial attack since freshwater snails "ingest and utilize bacteria."

FEEDING ACTIVITIES: PHYSICAL AND CHEMICAL FUNCTIONS

In the case of trailing behaviors some component within the viscous matrix is the active substance carried in mucin. In feeding behaviors, the quality of the mucus itself delineates function. Thus, viscosity, fluidity, adhesiveness, and porosity, for example, characterize the basic features of the secretion and its use in a variety of molluscan feeding activities.

Aside from the lubricatory functions played by mucins in molluscan feeding, there are several specific cases that represent the diversity of activities mucins play in feeding.

Cassid gastropods are noctournal predators, feeding on echinoids such as sea urchins. Salivary glands deliver a strongly acidic secretion that penetrates the test of the prey in under 10 minutes as it is grasped by the snail's foot (Hughes and Hughes, 1981). The acid, in conjunction with radular activity, penetrates the test so that soft parts may be ingested. When handling these spiny prey items, *Cassis tuberosa* (Linné) secretes a thick layer of "stiff mucus" from anterior pedal glands that flattens down the prey's spines and directs them away from the site of penetration (Hughes and Hughes, 1981). Under this mucoid layer, pedicellaria are detached, trapped, and thus "rendered harmless." *Cypraecassis testiculus* (Linné) similarly exudes a large amount of mucus over its prey and is thus able to climb onto it without harm from the spines (Hughes and Hughes, 1981). Houbrick and Fretter (1969) earlier described a thick mucous coat that surrounds the aperture of the gastropod prey, *Cymatium nicobaricum* (Röding), during proboscidial insertion.

The ciliary and mucus-net filter feeding behavior of *Crepidula fornicata* (Linné) is well known (Jørgensen *et al.*, 1984). Mucins are used to entrap, transport, and pelletize food particles (Werner, 1951, 1953). Similar systems are found in several prosobranch families (Capulidae, Turritellidae, Struthiolariidae) and have likely evolved independently (Hyman, 1967). In a burrowing struthiolariid gastropod of New Zealand, anterior and posterior tubes within the substratum are produced by the snail pushing its proboscis up and down, lining the forming canal with mucus (Morton, 1951). The snail is thus able to remain in a burrowed position and maintain ciliary induced feeding currents. Vermetid gastropods feed on plankton through combined use of mucous nets and ctenidial cilia (Hadfield *et al.*, 1972). *Hydrobia ulvae* (Pennant), a successful intertidal mesogastropod, attaches to water surfaces by a mucoid film (raft) during incoming tides and can use this film to trap additional food in the form of phytoplankton (Newell, 1962). Salivary gland mucocytes of adult *Helix aspersa* secrete neutral and acid mucins plus proteins involved in lubrication of foods and possibly preliminary digestion (Moreno *et al.*, 1982). Lubrication during ingestion is a common feature of mucins across the phylum Mollusca.

Bernard and Bagshaw (1969) found that the accessory boring organ (ABO) of the naticid gastropod *Lunatia lewisi* (Gould) is composed of two distinct epithelial regions: a peripheral area producing a "mucin" and a central mucoprotein producing region. Carriker and Williams (1978) and Carriker (1981) suggested that the mucoproteinaceous ABO secretion may contain chelating agents that aid calicum carbonate solubilization during drilling. At the very least mucoid secretions of naticacean and muricacean ABOs can act as lubricants and/or sealants to retain active secretions in place during boring activities (Carriker, 1981).

Some muricids apparently use paralytic agents produced by their hypobranchial gland to quell prey (Whittaker, 1960; Hemingway, 1978; McGraw and Gunter, 1972; Gunter, 1979). Presumably, hypobranchial glands of these predatory snails (e.g. *Thais haemastoma* Linné) secretes a mucus that can contain a choline ester carried to their prey in ciliary mantle currents. These esters have neuromuscular blocking qualities and can be released into the borehole produced by the ABO and radula. The proboscis is not inserted into the borehole but instead between the gaping valves of anaesthesized prey (Gunter, 1968, 1969). There have been no reports in the literature of similar activities in naticaceans but Carriker (1981) suggests that "Since these gastropods bind prey in large quantities of mucus during capture and manipulation prior to boring, the mucus itself, secreted presumably by pedal surfaces, could contain paralytic substances. These interesting possibilities call for attention."

Inhibitory or toxic secretions carried in mucins also occur in other classes of molluscs. Mebs (1973) describes the venom from *Octopus vulgaris* Cuvier (i.e., cephalotoxin) as a toxic proteinaceous substance, probably a glycoprotein. [Gennaro *et al.* (1965) also reported on a probable glycoprotein component of the salivary gland of *O. vulgaris* that may have some "salivary" or other "special function." In fact, Wood (1963) reported that the hundreds of thousands of eggs laid by female *O. vulgaris* are bound together with strands of mucus having their origin in the mouth of this cephalopod.]

SHELL FORMATION: POSSIBLE MATRIX AND TEMPLATE

During early stages of bivalve shell regeneration, Wada (1961, 1980) described small granules of "metachromatic organic matter" deposited between the shell and mantle of experimental (i.e., shell damaged) animals. Similar substances were described by Abolins-Krogis (1963) for *Helix pomatia* Linné and Uozumi and Suzuki (1979) for *Mytilus edulis*. These organic deposits contain a sulfated acid mucopolysaccharide with negatively charged sulfate groups that can bind calcium and thus induce calcium carbonate crystal nucleation (Watabe, 1983). Wada (1980), Crenshaw and Ristedt (1976) and Weiner and Hood (1975) suggest that granules of organic material can have surfically exposed carboxyl groups of aspartic acid that provide negatively charged sites for nucleation. Hillman (1968) suggested that the sulfated glycosaminoglycan produced by the outer fold of *Mercenaria mercenaria* (Linné) ". . . may play some role in shell deposition."

The organic matrix or conchiolin of molluscs, which contains mucin substances, has long been implicated in crystal nucleation. Wilbur and Saleuddin (1983) give "indirect support" of this as "the presence of protein and acid mucopolysaccharide at the sites of crystal formation," in addition to recalcification of previously decalcified "skeletal matrix" and calcium "binding by fractions of the soluble matrix." Mucins are particularly intimated in the more recent soluble-insoluble matrix theory of calcification. According to this theory, outlined by Crenshaw and Ristedt (1976), Crenshaw (1982) and

Wilbur and Saleuddin (1983), a soluble matrix portion stimulates calcification while an insoluble matrix portion inhibits calcification. Wilbur and Saleuddin (1983) suggest that adsorbtion of glycoprotein occurs on the "center of the exposed crystal surface where the concentration of free Ca is higher." Essentially, this would inhibit any additional surficial growth of the crystal. Free portions of the absorbed glycoprotein would, on the other hand, "concentrate Ca ions (and secondarily carbonate ions), initiating a new crystal through ionotropy" (see also Greenfield *et al.*, 1984). In both the soluble and insoluble components it is presumably a mucin (i.e., glycoprotein) that is the controlling feature. Kunigelis and Saleuddin (1984) recently reported that in *in vitro* studies of periostracum formation in *Helisoma duryi* Wetherby "Calcium binding by the organic matrix may occur either by constituent proteins or glycoproteins." This bound calcium can be attached to polysaccharide components of a glycoprotein as suggested by Crenshaw (1972), Wada (1980) and Wada and Fujinuki (1976). In this case, Kunigelis and Saleuddin (1984) hypothesized that it can be released "for precipitation as calcium carbonate or serve as a nucleation site for crystal growth."

In any case, importance of mucins in shell production is evident. The significance of mantle epithelium, the site of the supplying mucocytes, must not be overlooked. Joosse and Geraerts (1983) suggested that "It is not unlikely that mucus will change the diffusion of ions and water . . . and . . . alter the ionic gradients between the epidermal cells and the external environment." Calcium can be actively absorbed across the epidermis of *Lymnaea stagnalis* Linné as well as the mantle of the bivalves *Anodonta grandis* Say and *Amblema costata* Refinesque (van der Broght and van Puymbroeck, 1966; and Istin and Kirschner, 1968; Istin and Fossat, 1972, respectively). The corresponding ion transport properties of epithelial tissues and epithelial and extrapallial mucins almost certainly play an intimate role in regulation of calcification. Early evolutionary experiments with ion transport (calcium ion transport and concentration in particular) within an organic, perhaps mucoid, coat can well have played a role in the earliest shell formation.

REPRODUCTION: BEHAVIORAL DIVERSITY

Mucins play a role in some aspect of every phase of reproduction in molluscs from sperm transfer during copulation to egg layer formation and attachment of egg masses to the substratum, and in courtship behaviors of some terrestrial pulmonates.

Chace (1952) described an interesting aspect of mucin use in courtship behavior of some large limacid pulmonates. This behavior includes circling activities that involves consumption of the mate's caudal gland mucus [acid mucopolysaccharide, neutral mucosubstance and sulfomucin as described by Dalal and Pandya (1973) from the garden slug *Laevicaulis alte* (Férussac)] for ½-2½ hours. This is followed by the snails spiralling about each other and then descending together on a long (10-25 cm) thick, mucous cord where, suspended in air, they mate! Following mating the pair ascend the mucous cord, ingesting it as they rise. Caudal glands that produce mucus have been described in members of the Zonitidae as well as the Arionidae and Limacidae (Barr, 1928). Richter (1980) documented the use of caudal mucins in arionids for descent from vegetation (strands of mucus up to 1.75 m long in slugs weighing over 20 g), precopulatory ingestion, and antipredation.

A detailed review of the functions of mucins in spawn masses of prosobranchs may be found in Fretter and Graham (1962: Chap. 16).

In bivalves, inner demibranchs of corbiculaceans serve as marsupia for developing embryos. The bulk of the intralamellar epithelium lining the inner demibranchs is composed of mucocytes (Morton, 1977). Other secretory cells release small "globules" of material into the brood chamber which may function in nourishing embryos (Morton, 1977, 1982b). There is no report of the function of the inner demibranch mucocytes in brooding although Morton (1977) reports that secretory cells and mucous cells are hypertrophied in brooding adults of *Corbicula fluminea*. It is possible that these secretions might also be nutritive in function (although no evidence documents this or, for that matter, the notion that the products of other secretory cells are nutritive). More likely, the mucoid secretion in the chamber acts as a protective blanket surrounding and cradling developing young. The mucins could act to inhibit sudden environmental shocks within the chamber, absorbing physical impacts that might otherwise jostle the brooding adult into releasing its spawn prematurely. Since mucoid secretions play a similar role in other molluscs (albeit outside the adult), it is not unreasonable to assume that they have protective functions in a brood situation.

ANOMALODESMATAN MANTLE GLANDS: MUCINS IN PHYLOGENY

Mucoid secretions are particularly well known among bivalves from a relatively unknown subclass, the Anomalodesmata. Mucoid secretions, for instance, form the lining of the inhalent and exhalent respiratory and feeding tubes that penetrate the substratum from the burrowed condition of *Thracia phaseolina* Lamarck (Yonge and Thompson, 1976). Similarly, *Cochlodesma praetenue* (Pulteney) lines an inhalent tube with mucus. Particularly obvious among many of the members of this subclass is the possession of numerous small multicellular glands that line at least a portion of the mantle edge and produce an adhesive mucus. Allen and Turner (1974) first discovered these organs in some deep sea Verticordiidae and termed them "radial mantle glands." Prezant (1979a, 1981a) later found them in two of the three genera of marine lyonsiid bivalves. The secretion from these glands (in lyonsiids a glycoprotein-mucopolysaccharide complex) is released upon the periostracum and serves as a glue to attach extraneous particles (usually sand) to the outside of the shell. Because of this habit, Prezant (1979b, 1981a) termed the organs arenophilic radial mantle glands (or just arenophilic glands). In members of the genera *Lyonsia* and *Entodesma* [the third lyonsiid genus, *Mytilimeria*, is monotypic (i.e., *M. nuttalli* Conrad) and lacks arenophilic glands], the glands are modifications of the outer mantle fold. Both genera possess free-living bivalves that are either shallow sand burrowers (i.e., *Lyonsia*) or crevice dwellers along rocky shores or among tunicates (i.e., *Entodesma*). The mucoid attached sediment cover may play a role in stabilizing the weakly byssate members within the genus *Lyonsia* within shifting substrata or in protecting the thin shelled species of *Lyonsia* and juvenile members of the genus *Entodesma* from predators (esp. shell boring predators) (Prezant, 1981a). Based upon the location and structure of the arenophilic glands in the Lyonsiidae (i.e., glands located distal to the periostracal groove in species of *Entodesma* and proximal in *Lyonsia*) in conjunction with numerous other traits, Prezant (1981a,b) devised a phylogenetic scheme for the family. An early split in lyonsiid evolution was envisioned that entailed a divergence from a free-living *Lyonsia*-like ancestor to a *Lyonsia* branch and an entodesmid branch (latter including *Entodesma* and *Mytilimeria*).

Morton (1984) suggests that "radial mantle glands . . . (of *Clavagella australis* Sowerby) . . . are developed in the middle folds, not the outer folds as suggested by Prezant . . ." (latter from Prezant, 1979a). While it is almost a certainty that Morton found similar glands in *C. australis*, it is also equally certain that the Anomalodesmata are plastic enough that similar glands evolved in either middle or outer folds of the mantle edge. The glands in species of *Entodesma* open distal to the periostracal groove; this eliminates the possibility that the glands are modifications of the middle fold.

It is possible to hypothesize that arenophilic glands have evolved within the middle fold in anomalodesmatans other than the Lyonsiidae. Presently, we know of similar mucus secreting glands in the Periplomatidae (Morton, 1981a, Lyonsiidae (Prezant, 1979a, 1981a), Verticordiidae (Allen and Turner, 1974), Parilimyidae (Morton, 1982a), and Clavagellidae (Morton, 1984) (see Table 2). The only detailed report on these glands, however, is for the Lyonsiidae (Prezant, 1981a). While Morton (1981a) found arenophilic glands in *Periploma (Offadesma) angasai* Crosse and Fisher, no such glands were found in *Periploma fragile* (Totten) (Prezant, 1981a) indicating heterogeneity within a family outside the Lyonsiidae. Morton (1981a) suggests (for *P. angasai)* that the organs are "specialized glands evolved in some common ancestor of the Anomalodesmata and retained in a diverse series of descendants."

Similar glands found by Morton (1982a) in *Parilimya fragilis* (Grieg) also function in adherence of sand grains. Morton (1982a) suggests that ". . . the glands have an uneven distribution in the Anomalodesmata." It is also likely that they have an uneven structure and vary with regard to middle or outer fold origin and, if outer fold, site of secretion release. It is unlikely that such specialized glands arose separately in any major taxon within the Anomalodesmata. The Middle Ordovician stock of anomalodesmatans likely possessed an "anlage" of arenophilic glands, perhaps just a dense accumulation of mantle edge mucocytes (similar to that found in *Mytilimeria nuttalli* today although I suspect this bivalve has lost the glands over evolutionary time). The early mucocytes could have functioned to support and line burrows. With time the glands consolidated and became active in sediment adhesion to the shell. Extant members of the subclass that possess these organs could reflect a bifurcation from this early stock with mucocytes consolidating either within the middle or outer fold (Prezant, 1985).

Table 2. Arenophilic radial mantle glands: possession within Anomalodesmata.

FAMILY*	GLANDS	AUTHORS
Pholadomyidae Gray, 1947	-	Morton, 1980
Parilimyidae Morton, 1981	+	Morton, 1982a
Thraciidae Stoliczka, 1870	-	Prezant, 1981a
Periplomatidae Dall, 1895	+	Morton, 1981a
Laternulidae Hedley, 1918	-	Prezant, 1981a
Clavagellidae d'Orbigny, 1843	+	Morton, 1984
Lyonsiidae Fischer, 1887	+	Prezant, 1979a, 1981a
Pandoridae Rafinesque, 1815	-	Prezant, 1981a
Myochamidae Bronn, 1862	?	
Cleidothaeridae Hedley, 1918	-	Morton, 1974
Verticordiidae Stoliczka, 1971	+	Allen and Turner, 1974
Cuspidariidae Dall, 1886	-	Morton, 1982a

*Familial breakdown according to Morton (1982a,b)

Arenophilic glands are present in at least some members of several anomalodesmatan superfamilies (i.e., Pandoracea, Clavagellacea, Verticordiacea, Thraciacea). It is possible that the organs are primitive in origin and the anlage may well have been represented in early pholadacean stock of the Paleozoic. Present day pholadomyids lack arenophilic glands but the glands are, nonetheless, present in at least five families including the Clavagellidae. This is strong evidence for the primitive origin of the glands. As a primitive anlage in the Pholadomyacea the secretion could have had an early function as previously noted. Morton (1980) suggests that "The notion that the structure of primitive anomalodesmatans might be obtained from a study of *Pholadomya* must unfortunately be largely dismissed." He believes the specialized nature of this bivalve largely negates our ability to use it as a spring board in determining anomalodesmatans stock. *Pholadomya candida* Sowerby is extremely rare, perhaps now extinct, and unfortunately only a very few specimens have been examined [(Morton, 1980), for instance, had only a single specimen that was only partially sectioned; thus a large portion of mantle has been left unexamined microscopically.]. Prezant (1981a) showed that for at least the species within the lyonsiid genus *Entodesma* arenophilic glands are scarce and may indeed decrease in number as the animal grows. Thus, such glands could be present in the unexamined mantle edge of *P. candida* or in juveniles of this or other species examined. In either case, the presence or absence of these glands in an extant pholadomyacean does not discount the possibility of similar glands, or at least the anlage of a densely mucocyte packed mantle edge in stock Pholadomyacea. This might then have been the incipient evolutionary stage of present day arenophilic glands in the subclass.

The mantle edge of *Mytilimeria nuttalli* is densely packed with mucocytes. This is far from the discrete multicellular arenophilic mantle glands of their familial brethren *Lyonsia* and *Entodesma*. Glands of the latter two genera show distinct generic differences that can be ascribed to a bifurcated lineage. Loss of such glands in *M. nuttalli* could reflect its "new" habit of endosymbiontism within compound tunicates (stabilization, protection, nor camouflage are mandated as in free-living species of *Lyonsia* and *Entodesma*).

Bivalves with similar life styles to some of the Anomalodesmata with arenophilic glands might be expected to parallel their mantle development. This is not always the case. Members of the Pandoridae examined thus far (a family with members that approximate the sand dwelling habits of *Lyonsia* spp.) lack arenophilic glands. Similarly not all members of a given family possess arenophilic glands (i.e., not all Periplomatidae). This may be a bonus to the phylogenist in that strong parallelism may be absent. Thus, true lineages can be discerned with familial members lacking organs lost over their evolutionary development (as presumed for the lyonsiid *Mytilimeria nuttalli*). If this is the case, a detailed examination of these organs, in conjunction with sufficient comparable traits, could reveal distinct differences that show recent changes in structure, form, secretion, or site of release. Thus, slight modifications of an otherwise primitive trait could uncover important facts on the phylogeny of given anomalodesmatan families.

HYPOBRANCHIAL GLAND AND MUCIN GENERALIZATIONS

Bernard (1890) found that the primary secretion of the hypobranchial glands of *Nucella lapillus* (Linné) is mucus. These glands can be stimulated to secrete by the simple application of an irritant. Crofts (1929) injected irritating substances into the mantle cavity of abalone and stimulated an immediate response entailing mucoid streams pouring from the shell holes. Hypobranchial gland secretions have been associated with cleansing mantle cavities, protecting the molluscs by incorporation of a repellent, and various feeding activities. Considering the amount of research carried out on the hypobranchial gland and its secretion, it is surprising that so little is known of its function. Or is it? The knowledge of possession of a gland and the contents of that organ's secretion does not always allow development of an easy form-function relationship. Just because a gland produces a mucus, can we assume specific functions? Can we, for instance, conclude that because pedal glands of a gastropod secrete mucins, those mucins are lubricatory in function? It is reasonable to conclude that mucins released by salivary glands contain digestive enzymes involved in preliminary digestion? Can we assume that bivalve pallial secretions are involved in mantle cleansing activities or shell production? In fact, the answers to these questions will typically be yes, we can make these assumptions. That is the nature of mucoid secretions; they have a deeply etched, though diverse set of functions that allow generalizations based on gland structure and point of secretion release. The nature of mucoid secretions that allows them to have easily predicted functions is one of the basic features that also allows us to use these secretions in interpreting molluscan phylogeny to some extent. It is strongly possible that each presently known function of mucins has some "primitive" basis. Thus, we can surmise that digestion in molluscs always involved mucoid secretions, as did shell production, locomotion, respiration, osmoregulation, attachment, and many other molluscan activities. A basic feature of so many of the traits now used by molluscan phylogenists, which is omnipresent and ancient, is the mucoid secretion in one form or another. The secretion is primitive *but* the organ producing that secretion, and the subtle changes in that secretion itself (based on present functions), are almost certain to be apomorphs easily read in certain groups.

MUCIN TRENDS

Specific trends in mucin phylogeny within taxa are approached categorically by systematists, as any other character trait would be, but the ancient status of mucocytes adds the distraction of extremely long periods under evolutionary pressures. This feature, that is, the long history of these glands and secretions, allows speculation upon general trends in molluscan mucins. I will examine only one trend briefly.

The commonality of pedal mucins within the phylum Mollusca makes these secretions good candidates for a comprehensive analysis of mucoid trends. Figure 1 delineates possible trends within the phylum. If pedal mucins are an old trait (and there is no reason to doubt this), we can start with an ancestral mollusc that uses pedal mucins for locomotory lubrication and perhaps as an antidessicant (much as do some Turbellaria and Nemertinea). From this simple start, phylogenetic trends in molluscs have resulted in altered pedal mucins with functions in tube construction (several gastropods and bivalves), adhesion, trail specific behaviors, feeding, protection, and dispersal, for example. The adhesive functions of pedal mucins were probably an early phase of byssal development (a paedomorphic trait common to many bivalves). It is a short step from simple pedal adhesives to larval cements and adhesives of some ostrieds and pectinids. The trail of mucus left by most gastropods has evolved functions in inter and intraspecific trail following

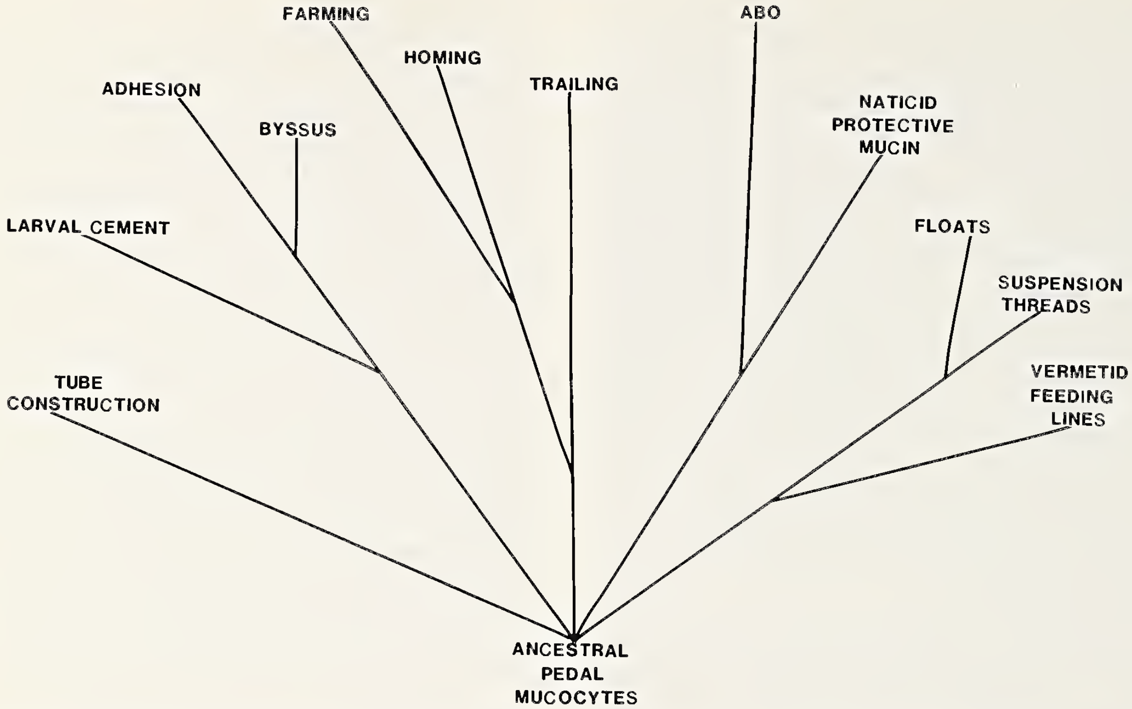

Fig. 1 Schematic delineation of molluscan trends in pedal mucins. The diagram does not denote any specific phylogenies but does signify possible trends in function derived from pedal mucocytes.

qualities. Homing behaviors (via trail following) has even taken on important qualities in food adhesion and microalgae growth in some grazing limpets. Pedal mucins of muricid gastropods have been "molded" into the highly efficient ABO. Pedal mucins of some cassids are active in protecting the snails. Some hydrobiid snails use pedal mucins for suspension in the water column, and formation of floating mucoid mats. This has been taken to an extreme in *Ianthina* with its mucoid bubble raft allowing it to drift in the same environment with its prey. Sessile vermetids no longer employ pedal mucin lubricants but instead use pedal mucins to entrap planktonic prey on sticky lines. Pedal mucins offer a simple framework for speculation of developmental and phylogenetic trends. Trends in pedal mucin glands need entail only simple morphologic changes to develop the diverse structures we see today. While possession of pedal mucocytes is certainly primitive, modified behaviors in association with changes in gland form offer ideal character traits to delineate apomorphs for phylogenetic interpretations.

"PREADAPTIVE" MUCINS AND EVOLUTION

In his delightful essay, "The problem of perfection, or how can a clam mount a fish on its rear end?", S. J. Gould (1977) discussed his "favorite example of an awe-inspiring adaptation," the "ersatz fish" of the several species of the freshwater mussel *Lampsilis*. The evolutionary development of this piscine mimic has followed a route of preadaptive development. While, as Gould argues, the term "preadaptation" is a poor one that conjures up a variety of misinterpretations, it is a term that describes a homologue that may have previously (phylogenetically) functioned in a different manner than the extant example. In his example, Gould argues that there are a variety of "stages" exhibited in freshwater mussels that represent developmental levels (a recapitulation?) leading to the fish lure. Kraemer (1970) suggested that the flapping tissues evolved to aerate brooded larvae or maintain released larvae in suspension. In either case the movement was a "preadaptation" to the fish lure movements. Might a variety of mucoid secretions also be "preadaptive" features?

The ctendial mucocytes of the Asiatic bivalve *Corbicula fluminea* offer a prime example of a "preadaptive" feature. During early stages of corbiculid phylogeny, ctenidial mucocytes undoubtedly played a role similar to that of most "eulamellibranchs" today; particle movement (food and debris-feeding and cleansing currents), facilitating respiratory gaseous exchanges, and perhaps some osmoregulatory function. With the development of brooding, in these often lotic bivalves, ctenideal mucus could have developed some function in marsupium maintenance and brood protection. Eventually mucoid secretions parlayed a new and important function in dispersal. The mucoid secretion, originally a typical ctenidial feature active in molluscan gill functions, now is active in brooding young and also in acting as a drag line secretion to carry small clams to new sites (Prezant and Chalermwat, 1984). The same mucoid product has several functions, the latter being relatively recent in acquisition. This modified use of ctenidial secretion could give *C. fluminea* an adaptive edge in dispersal among the freshwater bivalve molluscs.

Stasek and McWilliams (1973) speculate on a turbellarian-like ancestor for molluscs. Turbellaria are well known for their slime-producing qualities. They deposit mucous trails as they move over the substratum, entangle prey with mucus, remain attached in turbulent environments with a mucoid-glue, and even raise themselves on a transparent mucoid layer (*Convoluta*) off the substratum to allow reflection of sunlight to their ventral symbiotic algae (Fraenkel, 1961). In ancient stocks, a turbellarian mucoid sheath could have formed the nucleation or impregnation site for deposition of tanning elements, leading to periostracum and finally to shell. Von Graff (1904-1908) described "Schleimdrüsen" cells in acoel turbellarians that produce a homogeneous, viscous mucus. He further described a second mucocyte that produces formed secretory products (i.e. "Drüsen mit geformten Secreten") that include rhabdite-forming cells. Von Graff suggests that the latter mucocytes are modifications of the "Schleimdrüsen." Pedersen (1965), describing mucous gland cells of the acoel *Convoluta convoluta* (Abildg.), found two basic groups of mucocytes (based on fixed organisms), anteriodorsal mucocytes (with rod-like secretory granules) and posteroventral mucocytes (with small spherical or small rod-like secretory granules). Both cell types are basophilic, alcianophilic, metachromatic, PAS positive, and contain carboxyl, sulfate, and free hydroxyl groups. These are typical mucous components found interphyletically. There is good reason to suspect that the basis of these secretions, i.e., the mucoid matrix, is ancient in its origin.

Simkiss and Walter (1977), using epidermis from *Helix aspersa*, found that this tissue is "a relatively permeable structure." They found, in fact, that calcium can move bidirectionally across the pedal epidermis. Kwart and Shashoua (1957) produced a snail mucous model analogue that depended upon two polyacidic fragments linked by calcium ions. The calcium, however, would first have to penetrate the mucoid cover. Concentrations of calcium in the mucoid cover, preceeded or followed by a polymerization event, could indeed be an early stage of periostracum or shell of molluscs.

SUMMATION AND FUTURE RESEARCH

Hamilton (1967) has assumed that when a group of organisms penetrates a new ecological province and hence a new "adaptive zone" there could follow a rapid series of speciations and divergences into new ecological zones. Considering the initial protective functions of mucins, functions certainly necessary in the earth's early environments, it is easy to comprehend the continued successful use of invertebrate and vertebrate mucins. Gradual chemical changes of the small protein and carbohydrate portions of this basically watery substance could account for diversification in its functional uses. After all, mucus is 90.1-99% water in molluscs examined (Denny, 1983) and only the remaining solids dictate their functions and properties. Of these solids the inorganic salts play little if any role in determining the properties of the mucins; instead it is the small amount of carbohydrate and protein that do this (Denny, 1983).

The incredible diversity and yet fundamental similarity of mucoid secretions and secreting cells and tissues in molluscs underscores the potential importance they could have in molluscan systematics. Evolutionary trends are evident in their distribution, taxonomic affinities, and behavioral analogies. Trends and paths taken by anomalodesmatan bivalves, for example, indicate the importance of mucins in lifestyle and evolutionary development. The early molluscan stock of this subclass certainly had mucocytes well established in their morphologies. Mantle, gut and epithelial mucins diverged from the early stock to produce recent anomalodesmatans capable of attaching extraneous particles to their shells, channeling water currents through mucus lined tubes, binding food and feces with mucus, and cleaning mantle cavities of mucus trapped debris.

Since no fossil records exist, it is impossible to discern specific phylogenetic trends in secretory tissues. Interpretation of evolutionary histories thus has to be based on extant sequences of complexity and on assumptions of parallel functions and structures in extinct species found to have similar preserved parts. Assumptions must also be made concerning secretions from these tissues and of their functions. Can we assume that naticids that are now extinct also used mucus to enwrap their prey as do some of their extant counterparts? The likelihood that this was so is great; it is improbable that such complex activities are recently evolved.

The ubiquitous nature of mucocytes also makes interpretation of their phylogenetic significance difficult because of the obvious problem of convergence. Evolutionary shifts in form and function are easily envisioned when we scan the diversity of forms within taxa and the numerous "intermediate" forms between specific taxa. For example, the mantle edge of *Mytilimeria nuttalli* is dense with randomly dispersed mucocytes, quite distinct from the discrete multicellular glands of their familial relatives in the genera *Lyonsia* and *Entodesma*. Glands of species of *Entodesma* and *Lyonsia* also show distinct differences, easily accounted for by their lineage and habitats. Molluscs, however, tend to "solve" similar problems in similar ways. For example, arenophilic glands of lyonsiids are clearly elaborated mucous glands that serve to release an external glue for adhesion of extraneous particles on the shell (Prezant, 1981a). Sand forms a tight adhesive coat over the shell of *Samarangia quadrangularis* Adams & Reave as well (Clench, 1942), but it is unlikely that arenophilic glands are present in this venerid bivalve. Instead, as with the xenophorans, foreign matter is incorporated within the calcereous shell (Morton, 1985). External coats serve to increase shell weight, shell sculpture, and shell thickness. These in turn increase stability in the substratum and act as antipredation devices. Fossil records of sand covered bivalves must be interpreted with care with reference to possession of mantle mucous glands.

If we ascribe to the line of reasoning that features of commonality across a large spectrum of related taxa are probably primitive, we can assume mucins to be primitive. These plesiomorphs could have evolved early in ancestral molluscs and would have been available for evolutionary "experiments." The viscous nature of many mucins make them prime candidates to function in various roles in adsorption and absorption requiring only subtle changes in chemistry and physical properties to do so. Gradual functional changes could include the modification from cleansing and feeding mucociliary activities to using ctenidial mucins to bind and ensheath brooding young and to produce a drag-line for flotation dispersal in *Corbicula fluminea* (Prezant and Chalermwat, 1984). Similarly, previously dispersed unicellular glands of the mantle edge could initially have had a sediment binding, cleansing, feeding, or egestory function, then over evolutionary time condensed to the multicellular, club shaped arenophilic glands of lyonsiid bivalves (Prezant, 1979a, 1981a).

It is difficult to prove a monophyletic origin of mucin plesiomorphs. Any cladistic classification that we could devise would surely appear different from that of a traditional evolutionary systematist (c.f. Hennig, 1966). Sokal (1975) criticizes cladists as ignoring the "crucial aspect of macroevolution—the invasion of new adaptive zone." Mucin producing glands across animal phyla display convincing similarities in form and function. Are these all synonimies? Can we envision all mucocytes and mucin producing organs as arising monophyletically? Are there valid apomorphic mucoid producing structures?

The primitive nature of mucins has allowed evolutionary pressures to mold and shape a variety of forms of secretory structures and a variety of subtle chemical differences through various lineages. The potential of mucins to evolve into specialized secretions offers the phylogenist a strong tool. A large literature describes the variety of mucins and mucin secreting structures in the molluscs, but it is in the form of cursory comments within larger works or isolated papers on specific structures. With new techniques and methods of the "new synthesis" we must now include analysis of these mucous secretions and secretory structures. A literature review of any single molluscan family reveals an almost guaranteed reference to at least some mucus producing structures. Mucous glands, aside from structures such as the hypobranchial gland, are usually allocated to being a part of a larger organ analyzed for phylogenetic significance. It is time to examine the subunits of larger organs or tissues for the significance of individual mucus producing structures in evolution.

". . . life perversely tends to interdigitate all over, thus voiding man's clever schemes" (Croizat, 1958). Croizat's comment refers to schemes of phylogenetic sequencing. The "interdigitating" caused by convergence, parallelism, vicariance, and early adaptive radiations, all serve to complicate the work of the systematist. With limited knowledge of the evolution of mucins, a bewildering array of information on molluscan mucins but no distinct evidence of "mucins past," and the molluscan habit of "preadaptability," we can indeed devise many "clever schemes," ready for shattering as new data emerge. However, with the highly specialized secretory structures we find among mollusc mucoid glands today, in conjunction with the chemistry and behavior of their secretions, new data will emerge that will offer important clues to the phylogeny of molluscs.

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TOPICS IN MOLLUSCAN MINERALIZATION: PRESENT STATUS, FUTURE DIRECTIONS

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ABSTRACT

Recent advances are described in three areas of molluscan mineralization: (1) the influence of central ganglia on shell deposition; (2) the relation of mineralized granules to physiological functions; and (3) functions of the shell organic matrix.

Brains of fast-growing snails have been found to increase the *in vivo* shell growth rate of slow-growing animals and the rate of *in vitro* periostracum production by the mantle. Calcium carbonate and calcium phosphate granules have been observed to demineralize during shell repair at the time of glochidial shell growth and during the intrauterine development of embryos. In each case, the granules are thought to provide a source of calcium. The source of stimulation for demineralization and the cellular changes involved are unknown. *In vitro* studies have shown that the shell matrix has the capacity to initiate crystal formation and to influence the orientation of the growing crystals.

Needs for investigations in each of these three areas as well as transepithelial transport are emphasized. Possible directions of future research are pointed out.

In discussing mineralization in molluscs, I have selected four topics relating to calcium carbonate deposition and dissolution. These are: (I) ion movements; (II) the role of organic matrix in crystal formation; (III) mineralization and demineralization of granules; and (IV) neurohormonal influences in control of mineralization and demineralization. I shall briefly summarize some current studies in these four areas and then mention directions that might be considered in extending our information. The mechanisms involved in each area must eventually be clarified for our understanding of molluscan mineralization.

Table 1. Molluscan mineralized structures. (Tompa, pers. comm.)

shell
epiphragms
hinge ligaments
radula, teeth
resilium
calcified gill bars
eggshells
egg cases
spermatophore walls
burrow linings
penial hooks, plates, stylets, rods, pallets
byssal complex
eye lens
spicules
statoliths
darts
gizzard plates, beaks
calcified arteries
renal deposits
intracellular granules
extracellular phosphate granules of gills and inner shell surface

Table 2. Molluscan biominerals (after Lowenstam and Weiner, 1983).

Mineral types	Numbers occurring
Carbonates	5
Phosphates	7
Halides	2
Iron oxides	4
Silica	1
Oxalate	1
	20

First, a general point concerning molluscan mineralization. The scope of mineralizing activities in molluscs, as indicated by the variety of mineralized structures and the number of biominerals deposited, is striking. Table 1 lists 27 structures that are mineralized. In forming these structures, molluscs have deposited 20 minerals (Table 2), more than any other phylum (Lowenstam and Weiner, 1983). As we know very little of the mechanisms which result in this mineral specificity, this should prove a very interesting area of experimentation which will need to be carried out at the cellular level (Wilbur, 1984).

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I. ION MOVEMENTS IN $CaCO_3$ DEPOSITION

Four features of mineral deposition and demineralization are immediately evident from equations (1) and (2).

$$CO_2 + H_2O \overset{\text{carbonic anhydrase}}{\rightleftharpoons} HCO_3^- + H^+ \quad (1)$$

$$Ca^{2+} + HCO_3^- \rightleftharpoons CaCO_3 + H^+ \quad (2)$$

1. Carbonic anhydrase catalyzes the formation of HCO_3^-.
2. HCO_3^- is the immediate source of CO_3^{2-}.
3. A proton is released for each molecule of $CaCO_3$ formed. If the proton is not eliminated, the reaction will not continue and $CaCO_3$ formation will cease.
4. As the animal forms acid (e.g., succinic) under anaerobic conditions, the $CaCO_3$ in shell previously deposited will be dissolved.

Figure 1 shows the relationships between the outer mantle epithelium, the extrapallial space, and the inner shell surface schematically. At present, the data on mechanisms concerning epithelial transport of Ca^{2+}, HCO_3^-, and H^+ are too few to give confidence in their general application. Ca^{2+} is probably actively transported into the extrapallial fluid through the cells of the outer epithelium against an electrochemical gradient (Sorenson et al., 1980), although movement through extracellular spaces is a possibility since calcium has been localized in the spaces (Saleuddin, pers. comm.). Presumably, Ca-Mg ATPase with energy provided by ATP would be the mechanism of active transport through the cells. HCO_3^- is thought to be actively transported into the extrapallial fluid as well (Wheeler, 1975). The elimination of H^+ could occur either by reacting with HCO_3^- to form CO_2 or by reacting with ammonia to form ammonium ion (Campbell and Boyan, 1976; Loest, 1979; but see Simkiss, 1976). Carbonic anhydrase could catalyze the reaction with HCO_3^- and facilitate the diffusion of CO_2 out of the extrapallial fluid (Wheeler, 1975; Gros *et al.*, 1976; Gutknecht *et al.*, 1977). If free phosphate were present in the extrapallial fluid (see Marsh and Sass, 1984), this may have the further effect of greatly facilitating H^+ diffusion (Gros *et al.*, 1976). When one considers the organic compounds present in extrapallial fluid and possible chemical influences of the environment on mineral deposition, the complexity is very much greater than figure 1 would suggest.

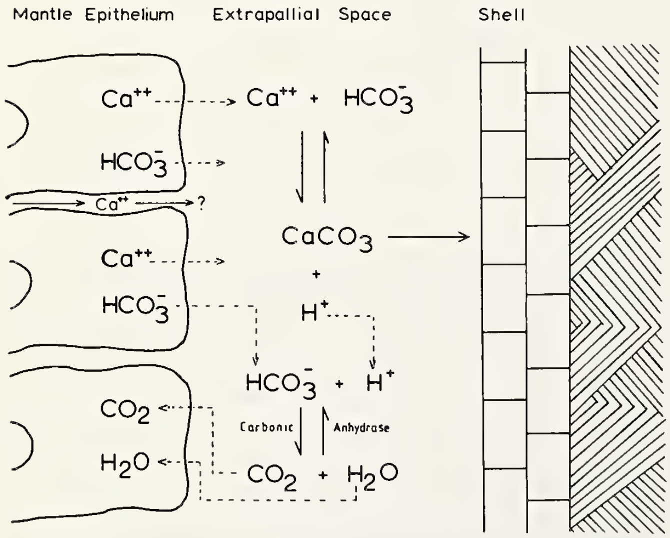

Fig. 1. Ion movements and reactions in shell formation. The diagram shows the compartments directly involved in $CaCO_3$ formation and deposition as shell. Reactions involving ion movements other than those indicated may occur but are yet to be investigated.

We shall need to give further attention to the proposed mechanisms of ion transport across the mantle epithelium mentioned above and to examine other possible ion movements and transport mechanisms, including a Ca/H antiport and an H^+-pump. For these studies, approaches used in the study of transmembrane ion movements relating to mineralization in aquatic plants may be applicable (e.g., see Rasi-Caldogno, 1982; Lucas, 1983; Walker, 1983; Borowitzka, 1984). Isolated mantle preparations arranged as membranes separating two solutions (e.g., Roer, 1979; Enyikwola and Burton, 1983) will be useful for such studies.

II. ORGANIC MATRIX AND CRYSTAL DEPOSITION.

Mineral in molluscs is always deposited in association with an organic matrix. This obviously means that in forming major mineralized structures, epithelia must secrete organic matrix in addition to transporting ions. Much has been written on the possible functions of matrix. These functions include: (1) inhibition of crystal growth and control of crystal size (Crenshaw and Ristedt, 1976; Weiner and Traub, 1981; Wheeler *et al.*, 1981); (2) initiation of crystal formation (Crenshaw 1972; Crenshaw and Ristedt, 1976; Weiner and Traub, 1981; Greenfield *et al.*, 1984; Weiner and Traub, 1984); and (3) control of crystal orientation in shell formation (Wilbur and Saleuddin, 1983; Bernhardt *et al.*, 1985).

A. Crystal inhibition

The matrix can be separated into a soluble fraction within the crystals and an insoluble fraction surrounding them. Take the soluble fraction, add it to a solution containing Ca^{2+} and HCO_3^- in which $CaCO_3$ crystals normally form, and crystal formation will be inhibited (Wheeler *et al.*, 1981). Extrapallial fluid is also inhibitory (Wilbur and Bernhardt, 1984). However, the inhibitory property can be removed by passage through a DEAE column which removes negative groups. Apparently, the extrapallial fluid inhibits crystallization by attaching its negative groups to the calcium of the crystal surfaces. Here is an enigma. Clearly, the soluble matrix and the extrapallial fluid permit crystal growth during shell formation. Perhaps, if the negative groups which inhibit by attaching to calcium on the crystal surfaces are saturated with calcium, the inhibition would disappear. A simple experiment should answer the question. If excess free calcium is found to abolish inhibition, then this would indicate that negative groups on compounds in the soluble matrix and the extrapallial fluid become inhibitory to crystal growth when the mantle cells are not transporting enough calcium to saturate these groups.

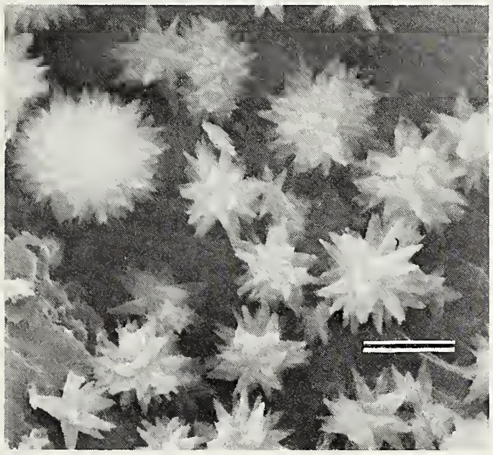

Fig. 2. Scanning electron micrographs of crystals deposited on *Nautilus* matrix during 3.5 hr incubation in metastable solution. Bar width = 500 µm. (Greenfield *et al.*, 1984).

B. Crystal initiation.

Evidence for crystal initiation by organic matrix is based on two types of evidence: calcium binding and crystallization of decalcified matrix *in vitro*. Analyses of soluble matrices have demonstrated calcium binding, presumably by ester sulfate groups ($-SO_4 \bullet SO_4-$) (Crenshaw, 1972; Crenshaw and Ristedt, 1976; Wada and Fujinuki, 1976; Wada, 1980), COO^- groups (Weiner and Hood, 1975; Krampitz *et al.*, 1976; Weiner, 1979; Krampitz, 1980), both, or a protein-isoprenoid hydrocarbon calcium binding system. (Samata *et al.*, 1980). These could be the same negative groups which inhibit $CaCO_3$ crystal formation. By binding Ca^{2+} and attracting CO_3^{2-}, these molecules are thought to initiate crystal nuclei which grow and become crystals (Weiner and Traub, 1981; Greenfield *et al.*, 1984).

Greenfield *et al.* (1984) have examined the capacity of the decalcified shell matrix of *Nautilus* to initiate crystal formation in a metastable inorganic solution. Crystals were formed in large numbers on the soluble matrix (Fig. 2) and in smaller numbers on the insoluble matrix. Bernhardt *et al.*, (1985) have also found that the matrix of *Argopecten* shell would become mineralized in an inorganic solution similar to sea water.

C. Crystal orientation.

Examination of the shell matrix of a number of species by x-ray and electron diffraction analysis (Weiner

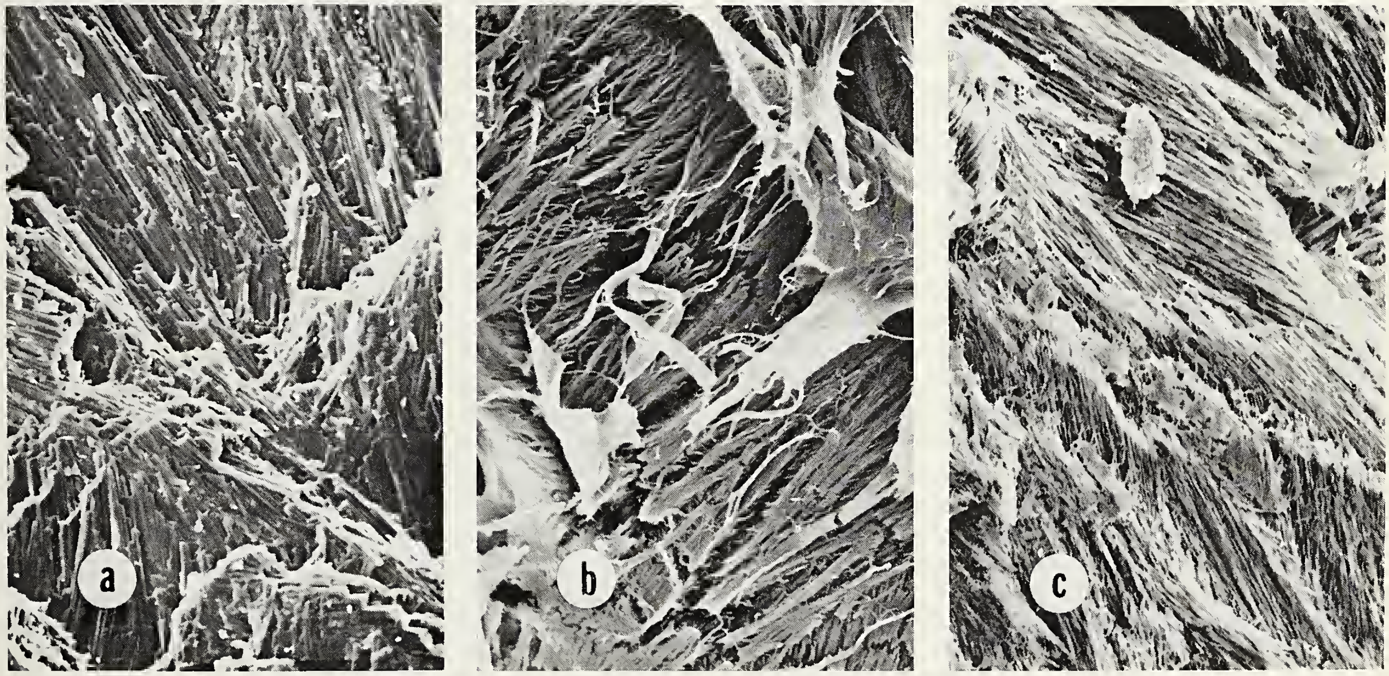

Fig. 3. Remineralization of shell of *Argopecten irradians* (Lamarck) Bernhardt *et al.*,1985). a. Elongate lath-like calcitic crystals of normal shell. Note parallel orientation of crystals. The angle of orientation differs somewhat among layers. The structural type has been termed regular foliated. Fracture surface about 2 mm. from shell edge. b. Decalcified shell matrix from a fractured shell. Note that the contiguous elongate matrix fibers are parallel in any given shell layer. The orientation of the matrix fibers differ somewhat among layers, as in the case of the elongate crystals. c. Remineralized matrix of a fractured shell. The crystals are elongate and parallel as in a., but differ from normal crystals in detailed structure. The mineral type is calcite, as in shell of this species. Horizontal field width = 150 µm for a, b and c above.

and Traub, 1981; Weiner *et al.*, 1983) has shown that the axes of the crystals of nacreous shell are aligned with the insoluble matrix. It was presumed that the molecules of the soluble matrix were oriented on the insoluble matrix and could induce crystal formation and control crystal orientation.

We have been able to demonstrate that decalcified matrix of *Argopecten* shell does orient crystals induced in an inorganic recalcification solution (Bernhardt *et al.*, 1985). Figure 3 shows the normal crystals before decalcification (a), the decalcified matrix (b), and the crystals which formed *in vitro* (c) during two days or longer. It is clear that in this species the matrix induces crystals having the same orientation as the fibers of the organic matrix. Presumably the soluble matrix had been removed during decalcification. If so, the insoluble matrix was directly responsible for both the nucleation and the orientation of the crystals. It is possible, of course, that some soluble matrix remained bound to the surface of the insoluble matrix and accounted for nucleation and crystal orientation. This possibility is supported by probe analysis demonstrating that the amount of calcium deposited during remineralization is far less than that present in the original crystals. Experimentation of this type should be extended to other species and classes to determine how general the relationship between matrix and crystal orientation may be. Further, the protein molecules of the shell matrix responsible for crystal initiation and orientation must eventually be isolated and their properties examined.

It is highly probable that soluble matrix is converted to an insoluble matrix by a phenoloxidase (Gordon and Carriker, 1980; Samata *et al.*, 1980) or, more specifically, a catecholase. If so, one might suppose that the molecular orientation of the insoluble matrix would be derived from the soluble matrix but with alterations due to enzyme action. However, this assumption is open to question in that the amino acid composition of the soluble and insoluble matrix is different (Meenakshi *et al.*, 1971). We must then ask what mechanism determines the orientation of the soluble matrix. Weiner and Traub (1981) assign the responsibility to the associated insoluble matrix, which would be the reverse of that just mentioned. Quite clearly, the cause-and-effect relation can be resolved only if the starting conditions are known.

It has been suggested that the internal surface of the periostracum is the starting point where the form of the first crystal layers deposited at the growing edge is determined (Taylor and Kennedy, 1969; Saleuddin and Petit, 1983; Wilbur and Saleuddin, 1983). The capacity of the periostracum to induce crystal nucleation and to control crystal form and orientation has not been examined experimentally but can probably be determined in an inorganic calcifying solution, as in Bernhardt *et al.*, (1985).

Whatever the factors determining the arrangement and orientation of the first crystal layer at the time of initial formation, the die has been cast for successive layers. Moreover, the axes of crystals in each succeeding layer will have a constant, or approximately constant, relation to the growth axes of the shell.

III. MINERALIZATION AND DEMINERALIZATION OF GRANULES.

In addition to shell and other major mineral structures, calcium may be present in considerable amounts as spherules or granules within intracellular vacuoles or extracellularly (Fournié and Chétail, 1982). The intracellular deposits are found in the connective tissue of the mantle, foot, visceral complex, albumen gland (Simkiss and Mason, 1983), blood vessels (Tompa and Watabe, 1976), gills (Silverman *et al.*, 1983), integument, and nervous system (Fournié and Chétail, 1984) of molluscs. Their common constituents are calcium, carbonate, phosphate, and magnesium. Other elements may be present in lesser amounts. These mineralized bodies merit attention because of their widespread occurrence in the Mollusca and other phyla (Simkiss, 1976) and because knowledge concerning their formation and demineralization is all too limited. They provide standby sources of Ca for maintenance of blood calcium and bicarbonate during periods of anoxia (Burton, 1983; Sminia *et al.*, 1977); for shell repair (Watabe *et al.*, 1976); for supplying the developing embryo (Fournié and Chétail, 1982); and perhaps in larval shell formation (Silverman *et al.*, 1983).

Granules, like shell, are dynamic mineral bodies exhibiting accretion, demineralization, and exchange. They can be considered one of the several interconnected compartments of molluscs (Fournié and Chétail, 1984). The intercompartmental fluxes will be governed by concentration gradients, membrane permeability, transport across barriers, and, in the case of the shell and granule compartments, by solubilizing influences of their immediate environments as well. Here I shall briefly mention three kinds of granules.

PHOSPHOPROTEIN SHELL GRANULES: Marsh and Sass (1983; 1984, pers. comm.) have described phosphoprotein granules containing calcium, magnesium, and phosphate in an organic layer on the central inner shell surface. The granules are present in some bivalve species but are absent from others (Fig. 4a). The particles can be demineralized in EDTA and partially remineralized in a solution containing calcium (Marsh and Sass, 1984). It has been suggested (Marsh and Sass, 1984) that these granules may provide an intermediate step for Ca^{2+} between the mantle cells and crystal deposition. Their organic phosphate and histidine residues may serve as a buffer against protons released in $CaCO_3$ deposition or in anaerobiosis (Marsh and Sass, 1983). The organic layer could perhaps retard ion movement from the extrapallial fluid to the inner shell surface and may account, at least in part, for the very slow rate of $CaCO_3$ deposition often characteristic of the central shell region. It is not known whether the granules and the organic layer are present at the shell edge where mineralization is relatively rapid. Their presence or absence there could be easily determined.

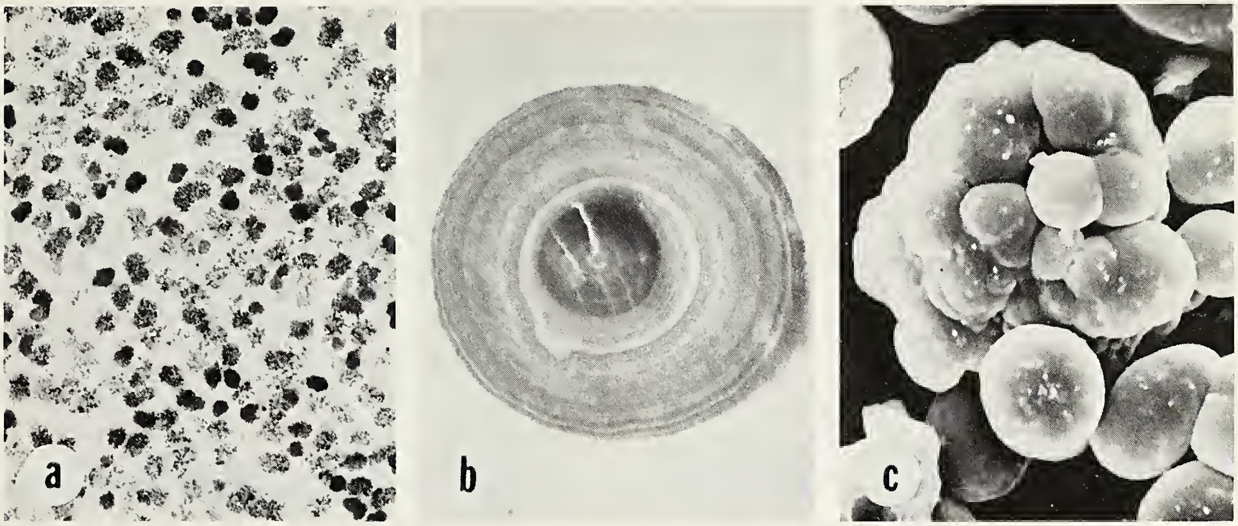

Fig. 4. a. Calcium phosphate granules of inner central shell surface of *Rangia cuneata* (Sowerby) (Marsh and Sass, 1983). Horizontal field width = 1.3 μm. b. Sectioned calcium phosphate granule from the gill of *Ligumia subrostrata* (Say) (Silverman *et al.*, 1983). Horizontal field width = 4.6 μm. c. Calcium carbonate granules from *Pomacea paludosa* (Say) tissues (kindness of N. Watabe). Horizontal field width = 1.2 mm.

CALCIUM PHOSPHATE GRANULES IN GILLS: A second type of granule, also calcium phosphate, has been described by Silverman *et al.*, (1983) and is found extracellularly in very large numbers in connective tissue of gills of freshwater bivalves (Fig. 4b). A most interesting feature of these structures is their disappearance at the time of growth of glochidia in the gills. Silverman *et al.* suggest (pers. comm.) that the spherules serve as a store of calcium used by glochidia as they form their calcareous shell. Indirect evidence for this is the finding that the amount of calcium demineralized in the granules is roughly equal to that of the shells of the glochidia.

INTRACELLULAR $CaCO_3$ GRANULES: A third type of granule (Fig. 4c) is deposited within cell vacuoles of several tissues. Structurally, it resembles the calcium phosphate granules of gills but is $CaCO_3$. Watabe *et al.*, (1976) observed that in shell repair in *Pomacea* the amount of calcium deposited during regeneration was approximately equal to the amount of calcium removed from the granules.

The demineralization of granules in the gills during development of glochidia and during shell repair raises the general question of the mechanism or mechanisms of demineralization. Suggested mechanisms include metabolic change resulting in pH decrease, catalysis by carbonic anhydrase, hormone action, and, in mammalian cells, calcium efflux mediated by inositoltrisphosphate (Berridge and Irvine, 1984).

The use of excised tissues containing intracellular mineral deposits and maintained *in vitro* would appear to provide a useful system for the study of demineralization and remineralization. Neurohormonal substances, metabolic compounds and other agents could be added to the cells and the accumulation or loss of mineral could be followed by means of radioisotopes.

IV. NEUROHORMONAL INFLUENCES IN CONTROL OF MINERALIZATION AND DEMINERALIZATION

Internal control of mineralization is evident in shell growth rate, crystal layering during shell formation, the shaping of the shell and other mineralized structures, the dissolution of granules mentioned above, and the mobilization of calcium during the ovulation of snail eggs possessing a shell of $CaCO_3$ (Tompa and Wilbur, 1977; Wilbur and Tompa, 1977) and in slug eggs containing calcium for the embryo (Fournié and Chétail, 1982). Our knowledge of internal control mechanisms associated with these mineralizing activities is very limited. Indeed, we know of only two internal mechanisms which alter the rate of mineral deposition: neurohormonal effects and catalysis by carbonic anhydrase.

Three findings demonstrate putative neurohormonal effects on shell growth rate. Geraerts (1976) demonstrated that cauterization of light green cells of cerebral ganglia in *Lymnaea stagnalis* Linné slowed shell growth. The decrease in shell growth rate could be corrected by implanting a corresponding part of the brain. Kunigelis and Saleuddin (1978) looked at brain effects on shell growth rate in another way. From populations of fast- and slow-growing *Helisoma* they removed brains or ganglia from the fast-growers, homogenized them, and injected the homogenate into slow-growers. This markedly increased the rate of shell growth. However, brains of slow-growers did not retard growth of the fast-growers.

The brain produces a specific effect on the mantle that relates directly to growth rate of periostracum (Kunigelis and Saleuddin, 1984). The mantles of slow-growing *Helisoma* elaborate periostracum *in vitro* at a slower rate than mantles from fast-growers. With the addition of the whole brain of a fast-grower to the mantle of a slow-grower, the rate of periostracum production was increased (Table 3). (See Joosse and Geraerts, 1983, for a comprehensive discussion of neurohormonal studies.)

Table 3. *In vitro* periostracum formation (after Kunigelis and Saleuddin, 1984).

Preparation	Periostracum formation mg. protein (± S.E.)
Slow-growing mantle	6.2 (± 0.2)
Slow-growing mantle & fast-growing brain	15.5 (± 1.2)

Since brain homogenates of fast-growing snails increase the rate of shell formation in slow-growing snails, two further effects of adding brain to isolated mantles can be anticipated: (1) an increased rate of transport of calcium and bicarbonate though the mantle epithelium and (2) a faster rate of secretion of organic matrix. These possible effects, together with a possible increase in the rate of matrix synthesis by the isolated mantle, will be worth attention in explorations of neurhumoral substances and mineralization mechanisms.

That carbonic anhydrase is a factor influencing shell growth rate was demonstrated by a reduction in rate by enzyme inhibitors (see Wilbur, 1972, for references). A finding of considerable interest concerns the carbonic anhydrase activity of the mantle in the fast- and slow-growing snails just mentioned. Carbonic anhydrase activity is highest in the fast-growers (Table 4) (Kunigelis and Saleuddin, 1983). Whether the brains of fast growers will cause greater activity of carbonic anhydrase in the mantle of slow-growing snails *in vivo* is inferred but yet to be determined. If increased carbonic anhydrase activity is observed, it will be necessary to determine whether the effect is due to activation or to enzyme synthesis.

Table 4. Mantle carbonic anhydrase and growth rate of *Helisoma duryi* Wetherby. (after Kunigelis and Saleuddin, 1983).

Growth rate	mm/day	carbonic anhydrase units per mg. protein (± S.E.)
Slow-growing	0.03-0.20	0.78 ± 0.03
Normal-growing	0.20-0.35	1.10 ± 0.04
Fast-growing	> 0.35	1.42 ± 0.06

Studies of neurohormonal effects during the past decade have laid a substantial groundwork for studies on the internal control of mineralization. Future experimentation can be expected to provide needed information on details of these mechanisms.

SUMMARY

Our discussion has concerned processes of mineralization at three levels: the whole organism, tissue and cell, and molecular. By way of summary, examples of research directions that are likely to be further developed at each level may be mentioned.

1. WHOLE ORGANISM. We can anticipate that the important studies on the effects of brain and ganglia on mineralization rate of the animal will be expanded. We have yet to explore the mechanisms by which nervous and hormonal systems may bring about the transfer of ions from mineral stores to eggs and embryos in the uterus or gills and to the repair sites of the shell. A three or four-step sequence would appear to be necessary.

a. Stimulation (for eggshell calcification, embryonic development and repair of shell) of a target site (uterus, embryos, mantle) where ions or mineral will be required for deposition.

b. Activation of chemical activity, which brings about demineralization and mobilization of mineral reserves (calcium-containing granules, shell, cellular accumulation), either as a direct result of target cell stimulation

or

c. mediated by a secondary message (neural or neurohormonal) produced by a central integrating mechanism (brain or remote ganglia).

d. Movement of ions to the site of final deposition.

The preferential localization of ions at these sites could result from an increased epithelial transport of ions and/or organic secretion by epithelium.

A second activity of the whole organism to be investigated is the control of shell structures consisting of layers of crystals. We do not know whether the mantle regulates the alternation of crystals and organic material which produces shell layering or whether a central mechanism provides the control.

2. TISSUE AND CELL. The functional stability of isolated mantles *in vitro* offers excellent experimental possibilities for studies of mantle functions *per se* and for investigations of neural and hormonal influences directly related to the shell-forming system. First priority should preferably be given to the design of experimental preparations which will permit measurements of ion movements, secretion, and metabolic effects. Such preparations can then be employed for studies of ganglion extracts, chemical agents, and direct nerve stimulation.

The uterus of gastropods which, like the mantle, transports Ca and secretes organic material may well prove another useful *in vitro* preparation for studies similiar to those suggested for the mantle.

In experiments directed toward clarification of the mechanisms of intracellular calcification and demineralization, it will be of considerable interest to apply ganglion extracts and other agents directly to calcifying cells *in vitro*.

3. MOLECULES. Isolation of proteins from shell matrix

and the testing of their capacities to initiate crystal formation and to orient crystal growth is becoming increasingly likely. Eventually, the compounds responsible for affecting the rate of mineralization will be isolated from the brain for testing on cells which form intracellular granules, on isolated mantles and uteri, and on the whole organism.

As a final point, I suggest that molluscan mineralization may well serve as a paradigm for all invertebrates in which a single layer of epithelium accomplishes the formation of an external layered skeleton of small crystals. This includes the bryozoa, brachiopods, molluscs, and crustacea.

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IDENTIFICATION OF BIVALVE LARVAE AND POSTLARVAE: A REVIEW OF RECENT ADVANCES

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ABSTRACT

Until the early 1960's, aids for identification of bivalve larvae and early postlarvae were based primarily on descriptions that relied on an error-prone "indirect approach" in which various possible ontogenetic stages were isolated from plankton and benthic samples. Development of laboratory-rearing techniques for culturing large numbers of larvae eliminated many of the ambiguities associated with the indirect approach and has permitted investigators to define certain early morphological features useful for discriminating species. The most useful of these features appears to be structures associated with the larval hinge apparatus (provinculum). These structures are visible under an ordinary compound microscope equipped with a high-intensity reflected light source, but because of their three-dimensional nature, a scanning electron microscope is necessary to photographically document the majority of these microstructural features. If results obtained from past and ongoing larval rearing studies can be combined and presented in a comprehensive, consistent and unified form, I envision that careful examination of the hinge region of larval and postlarval bivalves, when coupled with a consideration of gross shell morphological and morphometric features, can provide a means of unambiguously identifying many individual specimens at the species level.

Identification of bivalve larvae and early postlarvae has been the subject of extensive research since the late nineteenth century (Bernard, 1895, 1896a,b, 1897, 1898; Stafford, 1912; Werner, 1939; Jørgensen, 1946; Sullivan, 1948; Rees, 1950; Carriker, 1961; Newell and Newell, 1963; Loosanoff, Davis and Chanley, 1966; Chanley and Andrews, 1971; Lutz and Jablonski, 1978, 1981; Lutz *et al.*, 1982a,b). Early efforts focused on the isolation of various ontogenetic stages from plankton and benthic samples with an eye toward construction of continuous growth sequences in which the largest end-members could be positively identified based on the acquisition of certain adult morphological characters. Inherent problems associated with such "indirect methods" have been discussed by Loosanoff *et al.* (1966). The major advance in the field came in the early 1960's with development of extensive laboratory techniques for stimulating adult organisms of numerous species of bivalves to spawn and rearing the larvae of these animals under controlled culture conditions (Loosanoff and Davis, 1963). Such techniques allowed involved researchers to unambiguously describe the early ontogenetic stages of organisms positively identified at the species level (Fig. 1). This "direct" approach (see Loosanoff et al., 1966), in turn, permitted several investigators to define certain morphological features useful for discriminating individual larval and postlarval specimens at various taxonomic levels when such organisms are viewed under an ordinary compound (light optical) microscope (Loosanoff and Davis, 1963; Loosanoff *et al.*, 1966; Chanley, 1970; Chanley and Andrews, 1971; de Schweinitz and Lutz, 1976). Such features have included morphological characters such as length of the "hinge-line" of early ontogenetic stages (Fig. 2), the presence or absence of a byssal notch, larval eyespot (Fig. 3), or apical cilia (Fig. 4), and the size at which larval specimens develop a functional foot (pediveliger stage [Carriker, 1961]) (Fig. 5) (Chanley and Andrews, 1971; Culliney, Turner and Boyle, 1975; Turner

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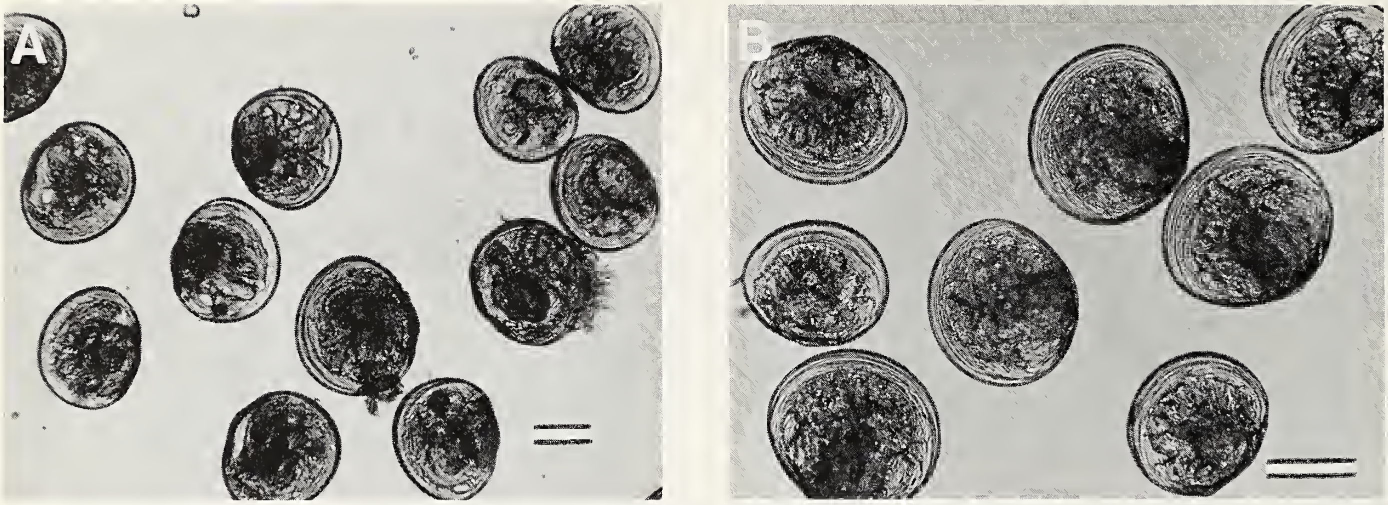

Fig. 1. Larval bivalves of various sizes reared from positively-identified adult organisms. (A) Larvae of the blue mussel, *Mytilus edulis*. Scale, 100 μm. (B) Larvae of the ocean quahog, *Arctica islandica*. Scale, 100 μm.

and Boyle, 1975, Lutz *et al.*, 1982a,b). Scatter diagrams depicting the relationship between shell height and length for larval and early postlarval specimens of individual species (Fig. 6) have also proved useful for identification purposes and several workers have suggested that the use of three-dimensional diagrams encompassing length-depth-height combinations for the early stages of individual species of bivalves (Fig. 7) provides an additional tool of even greater general utility for identifying such stages at specific levels. Differences in larval shape, color and texture have also been of assistance and perhaps the most useful identification aids from a practical standpoint have been photographic sequences in which the various larval stages from the straight-hinge stage through metamorphosis are depicted in a format similar to that shown in Figure 8. Unfortunately, as should be obvious from examination of the micrographs in this figure, subtle differences in shell shape are often the only distinguishing characteristics between various species and the consistent orientation of larval and postlarval specimens under the microscope presents serious technical problems.

More recently, various workers have employed scanning electron microscopy to describe in detail various micro- and ultrastructural characters of early ontogenetic stages of numerous living and fossil bivalve species, and have suggested that several of these characters may be diagnostic at the generic, or even specific, level (Culliney *et al.*, 1975; Turner and Boyle, 1975; Boyle and Turner, 1976; Booth, 1977, 1979a,b; Lutz and Jablonski, 1978, 1981; Lutz and Hidu, 1979; Chanley and Dinamani, 1980; Le Pennec, 1980; Lutz *et al.*, 1982a,b; Jablonski and Lutz, 1983) (Figs. 9-17). The characters that have received the most attention in this regard have been

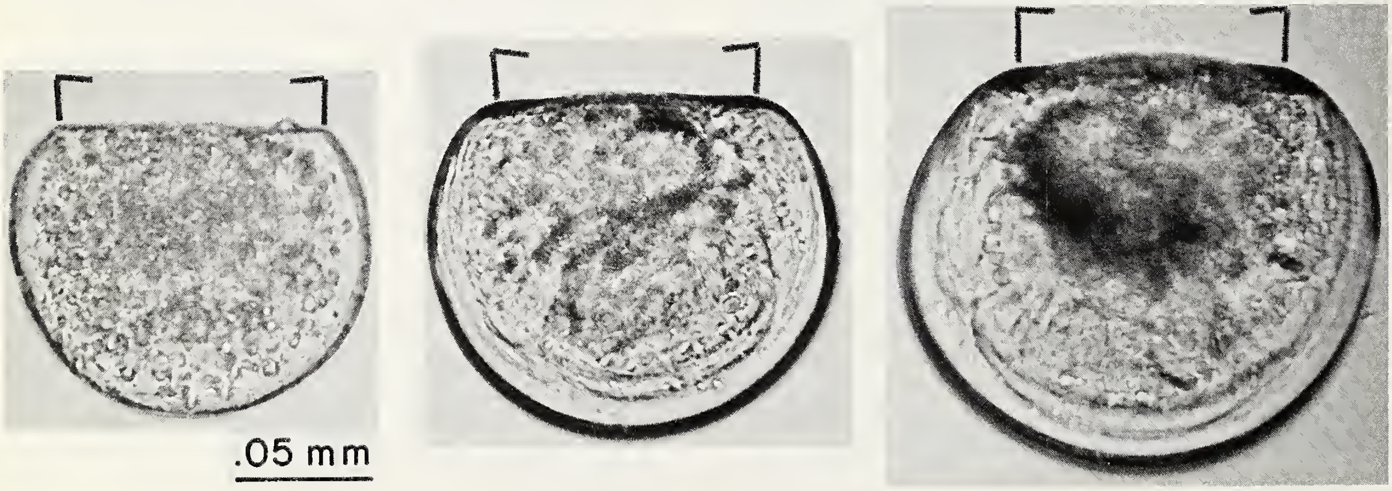

Fig. 2. Various stages of development of straight-hinge larvae of *Modiolus modiolus*. Brackets delimit the "hinge-line". Reprinted with permission from de Schweinitz and Lutz (1976).

structures associated with the larval hinge apparatus (provinculum) and the results of several recent studies have shown rather dramatic differences in the structure of both the larval and early postlarval hinge between various species (Le Pennec, 1980; Lutz *et al.*, 1982a,b). These structures are readily apparent under a scanning electron microscope (SEM) after living or preserved specimens have been immersed in a 5% solution of sodium hypochlorite (Rees, 1950) for approximately 10 minutes, rinsed in distilled water, air-dried, and appropriately mounted and coated on SEM stubs (see Carriker and Palmer, 1979; Clarke, 1981; Waller, 1981; and Lutz *et al.*, 1982a,b; for further methodological details). Such chemical treatment results in individual disarticulated shell valves such as those depicted in Figure 16, and it should be mentioned at this time that all of the structures seen in this figure and those described in the following discussion are visible (though not readily photographed due to their three-dimensional nature) under an ordinary compound microscope equipped with a high-intensity reflected light source. Examination of hinge structures of two closely related species such as those depicted in Figure 14 reveals a similar sequence of ontogenetic changes from the prodissoconch I stage through metamorphosis. Dentition is generally absent at the prodissoconch I stage and provinculum length and complexity usually increase throughout larval development. In the case of the family Mytilidae, for example, there is progressive lateral thickening of the provinculum and the number of hinge teeth in the prodissoconch II and early dissoconch stages increases significantly with both total shell and provinculum length (see Fig. 14). While differences such as those between the confamilial specimens depicted in Figure 14 are often subtle, they frequently can be quantified, at least at the population, if not the individual, level. Figure 15 summarizes results obtained after regression and quantitative comparison of a number of morphometric measurements made on various larval and early postlarval stages of the two species (*Mytilus edulis* Linné, 1758, and *Modiolus modiolus* [Linné, 1758]) depicted in Figure 14. While no significant differences were detected between slopes of regression lines in any of the four statistical comparisons (analyses of covariance) (Steel and Torrie, 1960; Sokal and Rohlf, 1969) run on the summarized data, highly significant differences (0.01 level) in regression line elevations were detected in each of the analyses. In addition, for a given number of teeth, no overlap was found between the range extremes in either provinculum length or total shell length of the two species (Fig. 15C,D), indicating that careful examination of the hinge morphology of prodissoconchs and dissoconchs should facilitate unambiguous differentiation of all early life-history stages of these two closely-related species (for further details, see Lutz *et al.*, 1982a). Such subtle, yet significant, differences are typical of those frequently encountered between confamilial species. In contrast, differences at the familial or superfamilial level are often striking. Figure 17 depicts a series of scanning electron micrographs of the hinge region of the larvae of the numerous species of bivalves depicted in Figure 16. The obvious differences in provinculum morphology provide a dramatic illustration of the potential usefulness of hinge structures for the identification of individual larval specimens.

Several workers have commented on the usefulness of a number of other larval and early postlarval morphological features apparent under the SEM that may be useful for discriminating various species. Since the classic studies of Bernard in the late nineteenth century (Bernard, 1895, 1896a,b, 1897, 1898), numerous researchers have commented on the presence of ligaments or ligament pits in "larval" specimens (Rees, 1950; Ansell, 1962; Loosanoff *et al.*, 1966; Chanley and Andrews, 1971; Bayne, 1976; Lutz and Hidu, 1979; Lutz *et al.*, 1982a). More recently, Lutz and Hidu (1979) have provided evidence that the primary ligament pit represents a postlarval, rather than larval, feature. This structure is readily apparent under the SEM (Fig. 18) and its position has been considered a diagnostic characteristic useful in superfamilial separation of early ontogenetic stages (for discussion, see Lutz and Hidu, 1979). Features associated with the external shell morphology of bivalve larvae and postlarvae, such as surface sculpturing and prodissoconch I

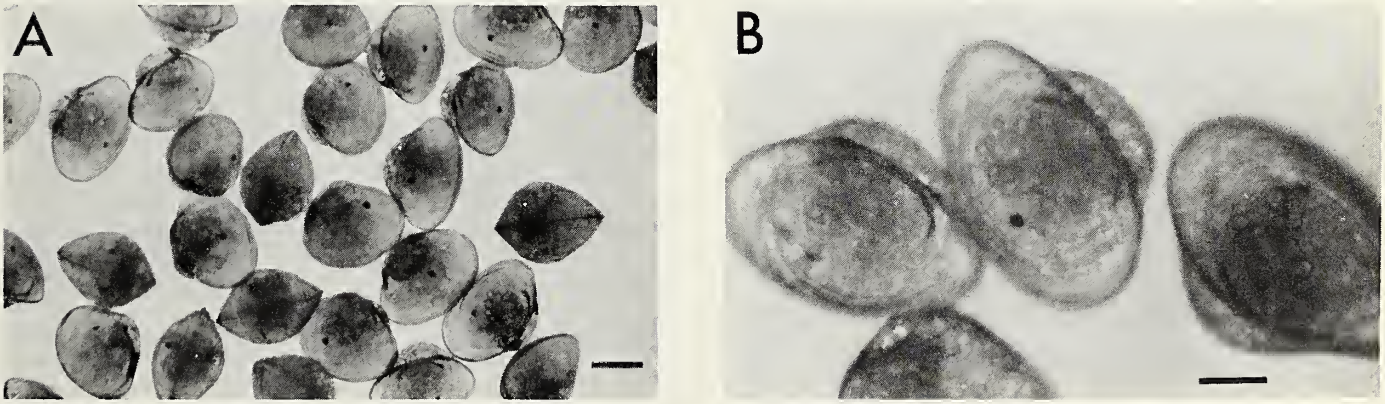

Fig. 3. Mature larvae of the common arc, *Arca noae* Linné, 1758. Note distinct larval eyespots (black dots). (A) Scale, 100 μm. (B) Scale, 50 μm.

and II boundaries, are often quite distinct in various species and may be of considerable assistance for identification purposes (Figs. 9, 10, 19, 20D-F) (LaBarbara, 1974; Lutz and Jablonski, 1978; Lutz *et al.*, 1982a). The morphology of the "soft parts" of the early stages of bivalves has received relatively little attention, although a few workers have presented micrographs of the velum and pediveliger foot of critical-point dried specimens and suggested that such structures may be of diagnostic value in systematic studies (Culliney *et al.*, 1975; Turner and Boyle, 1975; Waller, 1981; Lutz *et al.*, 1982a) (Fig. 20A-C).

Finally, while larvae of the majority of shallow-water temperate and tropical bivalves are characterized by a free-swimming, planktotrophic existence, many of the deeper, continental shelf species, as well as deep-sea and arctic bivalves, follow a nonplanktotrophic mode of development. Early ontogenetic stages of a number of these species are encapsulated and are seldom found in the water column (Fig. 21). Morphology of the capsule itself and size and shape of the encapsulated embryo or larva are often quite distinct and may be useful characters for identification purposes.

SUMMARY AND CONCLUSIONS

Our knowledge of bivalve larval and early postlarval shell morphology has increased in recent years to a point where we can now begin to synthesize results obtained to date into a comprehensive format that should facilitate identification of early ontogenetic stages of numerous bivalves isolated from both plankton and benthic samples. The format should include not only the "minimal information" recommended by Chanley and Andrews (1971, pp. 107-109) for "detailed descriptions of laboratory-reared bivalve larvae", but also detailed scanning electron micrograph sequences of the hinge structure and gross shell morphology of the various larval and postlarval stages, as well as selected micrographs of other diagnostic features, such as ligament pits and shell surface morphological features. Examples of such detailed scanning electron micrographic sequences for an individual species (*Arctica islandica* [Linné, 1767]) are presented in Figures 22-25. Presentation of results from past and ongoing studies obtained from laboratory-rearing studies in such a comprehensive format should provide an extremely powerful tool for the identification of larvae and early postlarvae of most species of bivalves, particularly those of commercial importance that have been the primary focus of the vast majority of laboratory culture studies conducted to date.

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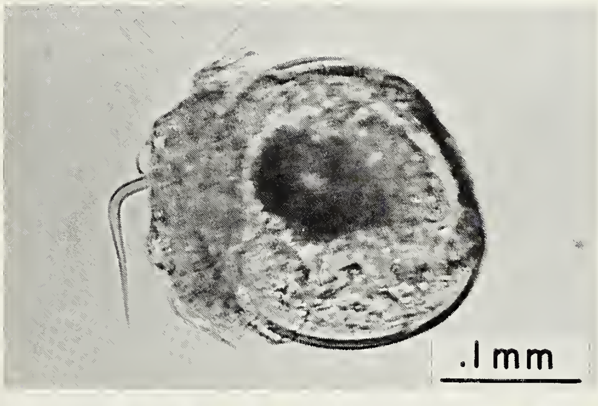


Fig. 4. Early umbo stage larva of the northern horse mussel, *Modiolus modiolus*, showing prominent apical flagellum. Reprinted with permission from de Schweinitz and Lutz (1976).

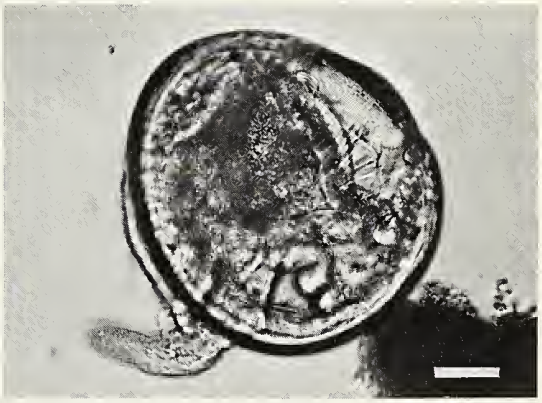


Fig. 5. Pediveliger larva of *Mytilus edulis* with an extended, functional foot. Scale, 50 μm.

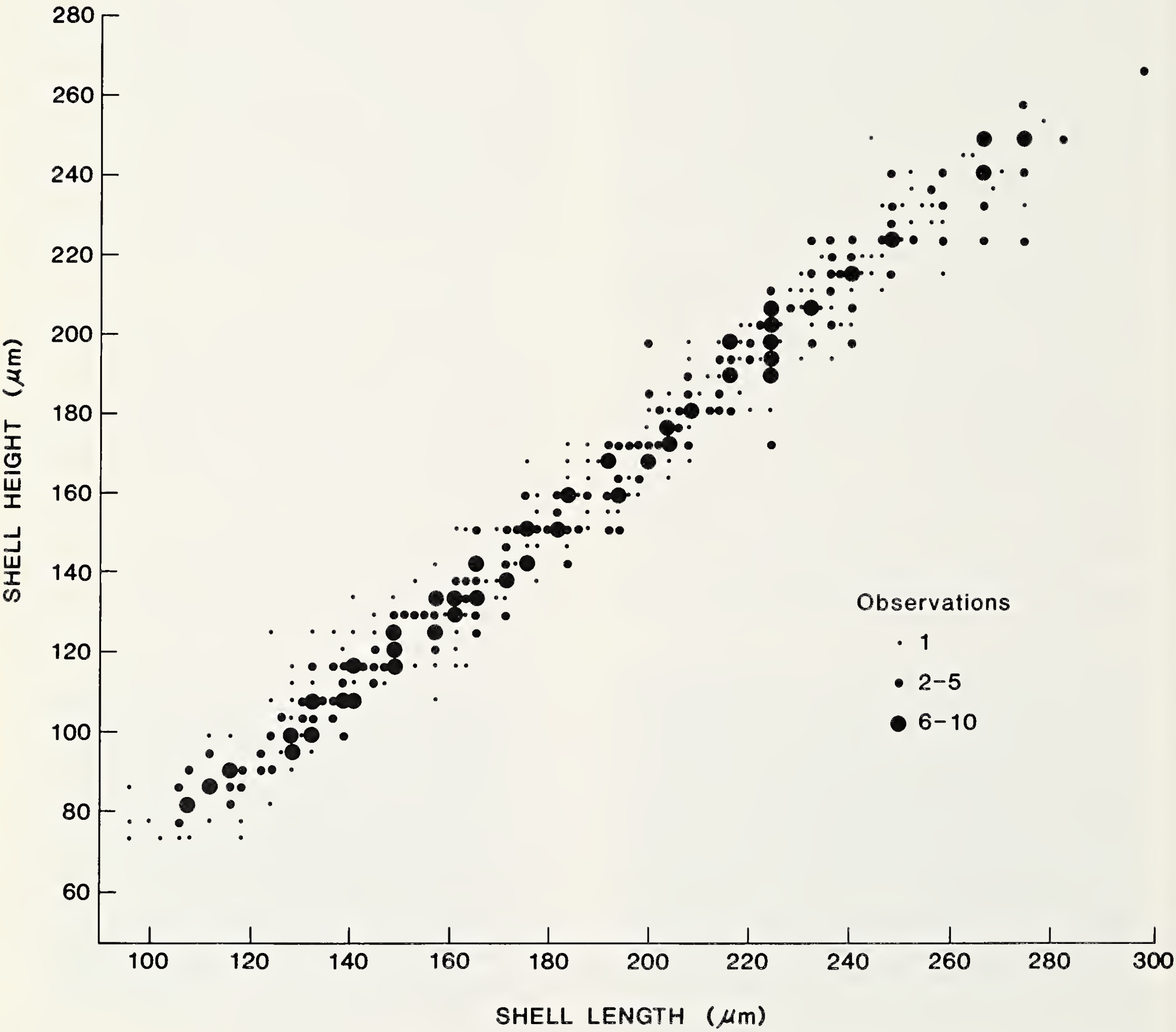


Fig. 6 Scatter diagram depicting the relationship between shell height (H) and length (L) for 821 larvae of *Arctica islandica* reared under a variety of culture conditions. The regression equation for the line of least squares is: H = 1.02L — 30.0. Reprinted with permission from Lutz *et al.* (1982a).

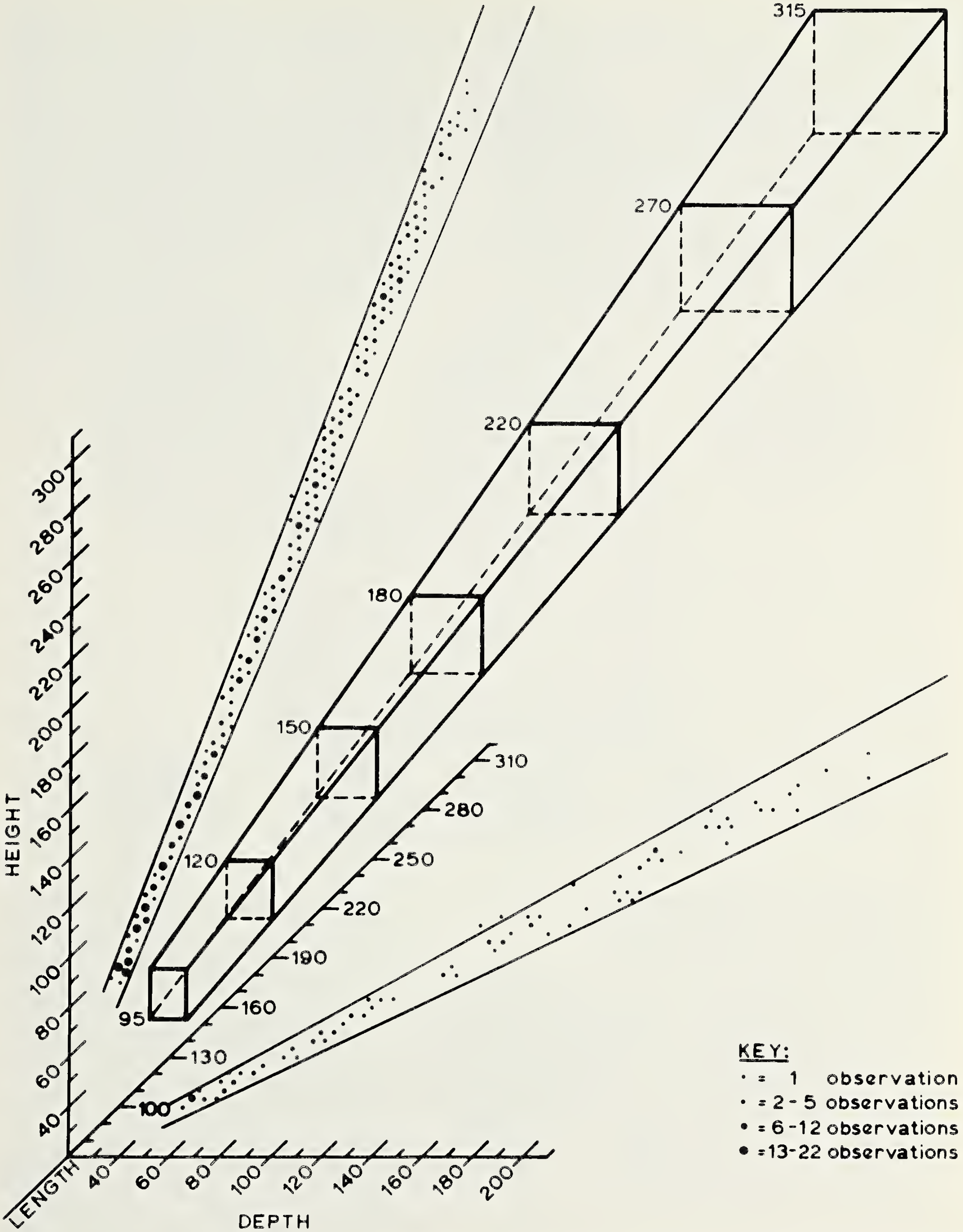


Fig. 7. Three-dimensional diagram depicting larval shell dimensions of *Mytilus edulis*. Height and depth coordinates run parallel to the length axis. Dots represent observed length-height or length-depth measurements. The polyhedron encompasses all possible length-depth-height combinations of *M. edulis* larvae. Reprinted with permission from de Schweinitz and Lutz (1976).

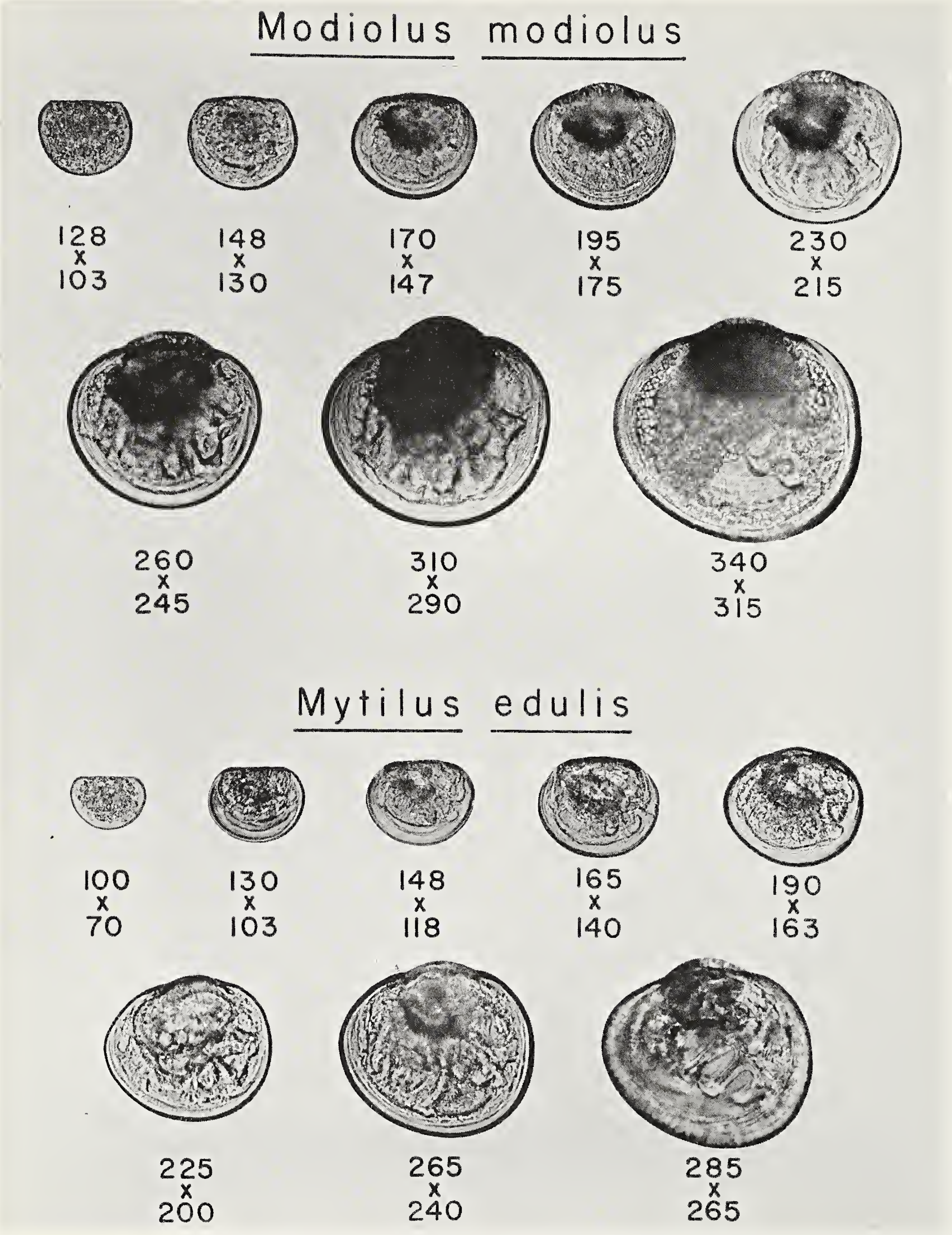


Fig. 8. Photomicrographs of the larvae of *Modiolus modiolus* and *Mytilus edulis* from the early straight-hinge stage to metamorphosis. Length and height dimensions are given in micrometers below the individual specimens. Larvae are oriented with the anterior end to the left. Reprinted with permission from de Schweinitz and Lutz (1976).

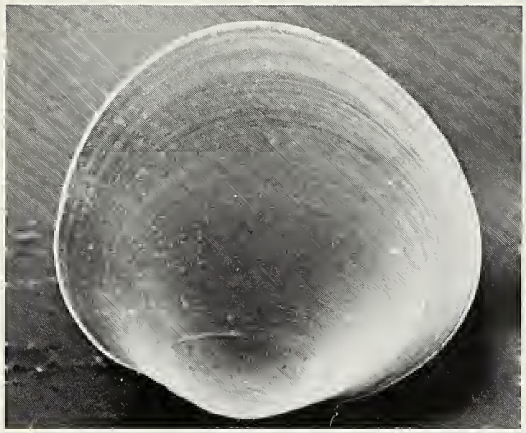


Fig. 9. Scanning electron micrograph depicting the external shell morpholoy of the left valve of a mature larval specimen of *Modiolus modiolus* with a shell length of 292 μm.

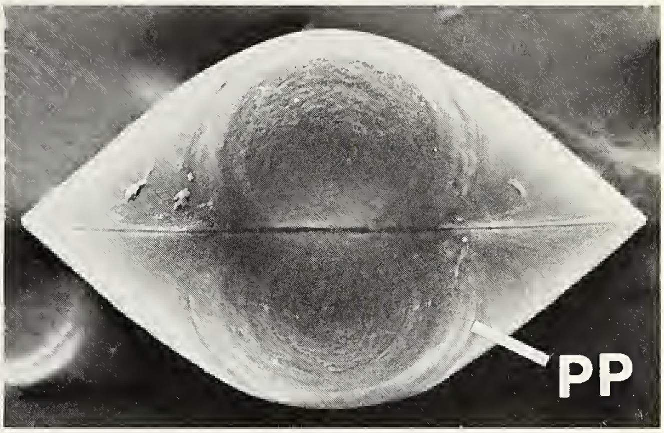


Fig. 10. Scanning electron micrograph of the dorsal region of an articulated larval specimen of *Modiolus modiolus* with a shell length of 290 μm. Note the distinct boundary (PP) between the prodissoconch I and II shell.

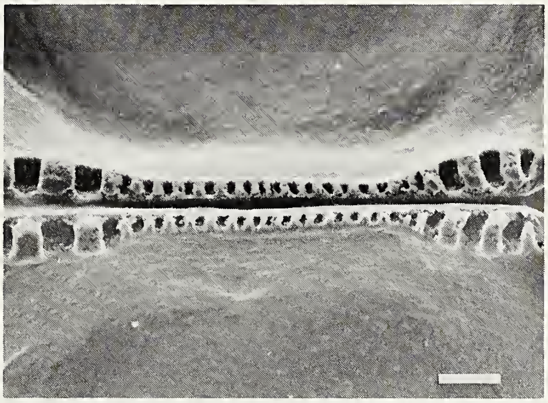


Fig. 11. Scanning electron micrograph of the hinge region (provinculum) of disarticulated shell valves of a specimen of the Atlantic ribbed mussel, *Geukensia demissa* (Dillwyn, 1817). Scale, 10 μm.

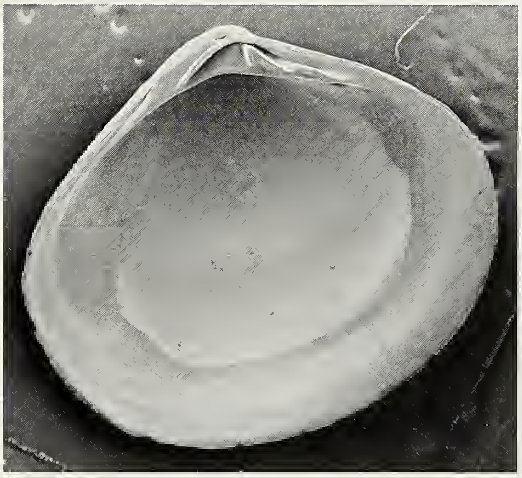


Fig. 12. Scanning electron micrograph of a disarticulated shell valve of an *Arctica islandica* specimen with a shell length of 4.0 mm. Reprinted with permission from Lutz *et al.* (1982a).

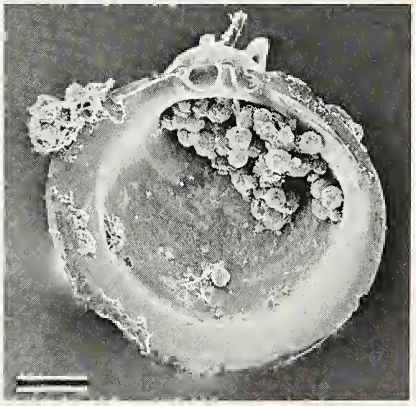


Fig. 13. Scanning electron micrograph of a disarticulated shell valve of a Cretaceous bivalve larva from the Monmouth Formation near Brightseat, Maryland. Larval dentition, length by height relation, and umbonal shape are characteristic of the family Pholadidae. Scale, 40 μm. Reprinted with permission from Lutz and Jablonski (1978).

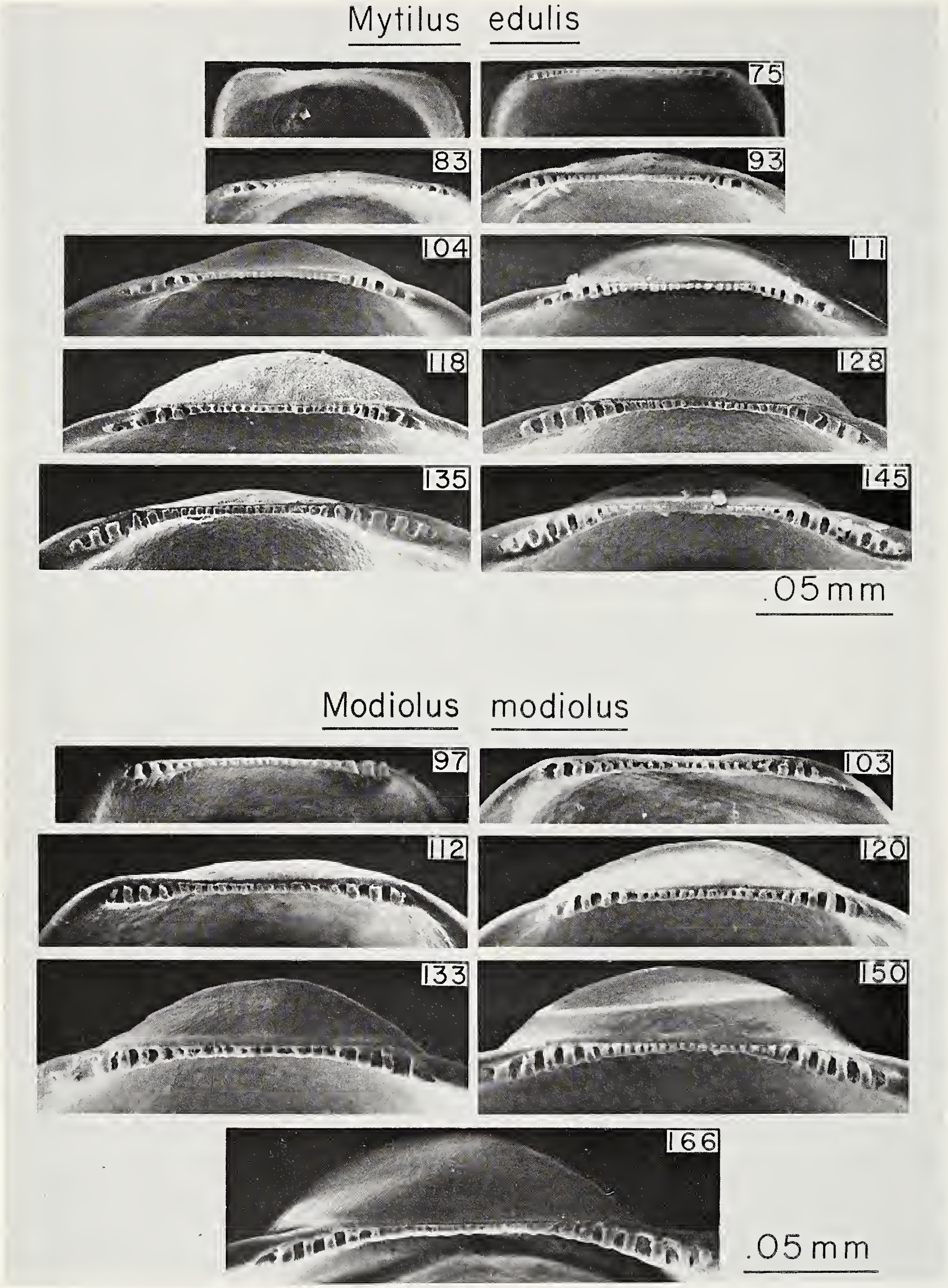


Fig. 14. Scanning electron micrographs of disarticulated shell valves of *Mytilus edulis* and *Modiolus modiolus* depicting hinge structures from the straight-hinge stage through metamorphosis. Numbers designate length of the provinculum in micrometers. Reprinted with permission from Lutz and Hidu (1979).

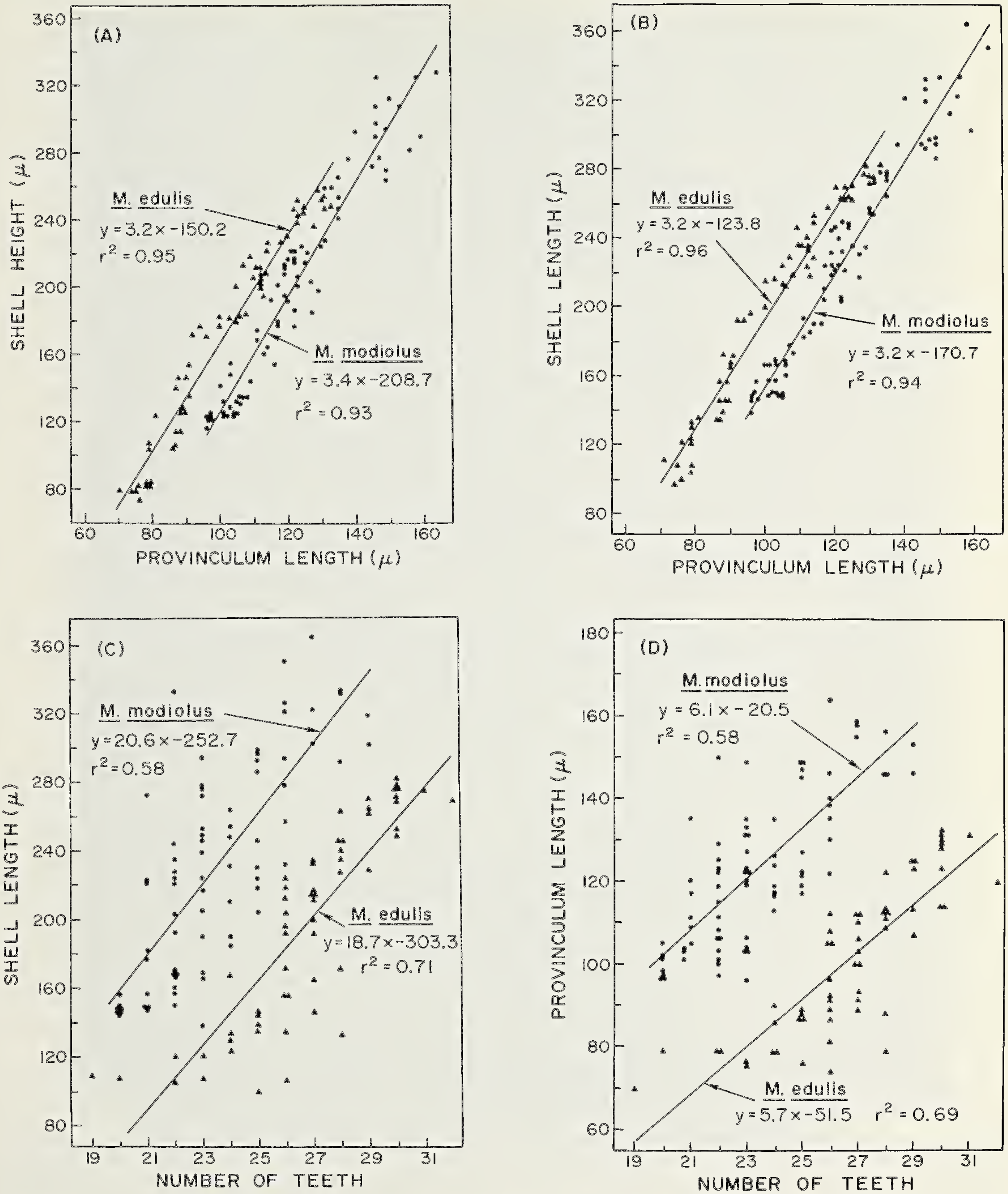


Fig. 15. Regression relationships between various shell characteristics of larval and early postlarval specimens of *Mytilus edulis* (▲) and *Modiolus modiolus* (●). Lines of least squares, regression equations and correlation coefficients are superimposed on each of the graphs. (A) Shell height *vs* provinculum length. (B) Shell length *vs* provinculum length. (C) Shell length *vs* number of teeth. (D) Provinculum length *vs* number of teeth. Modified from Lutz and Hidu (1979).

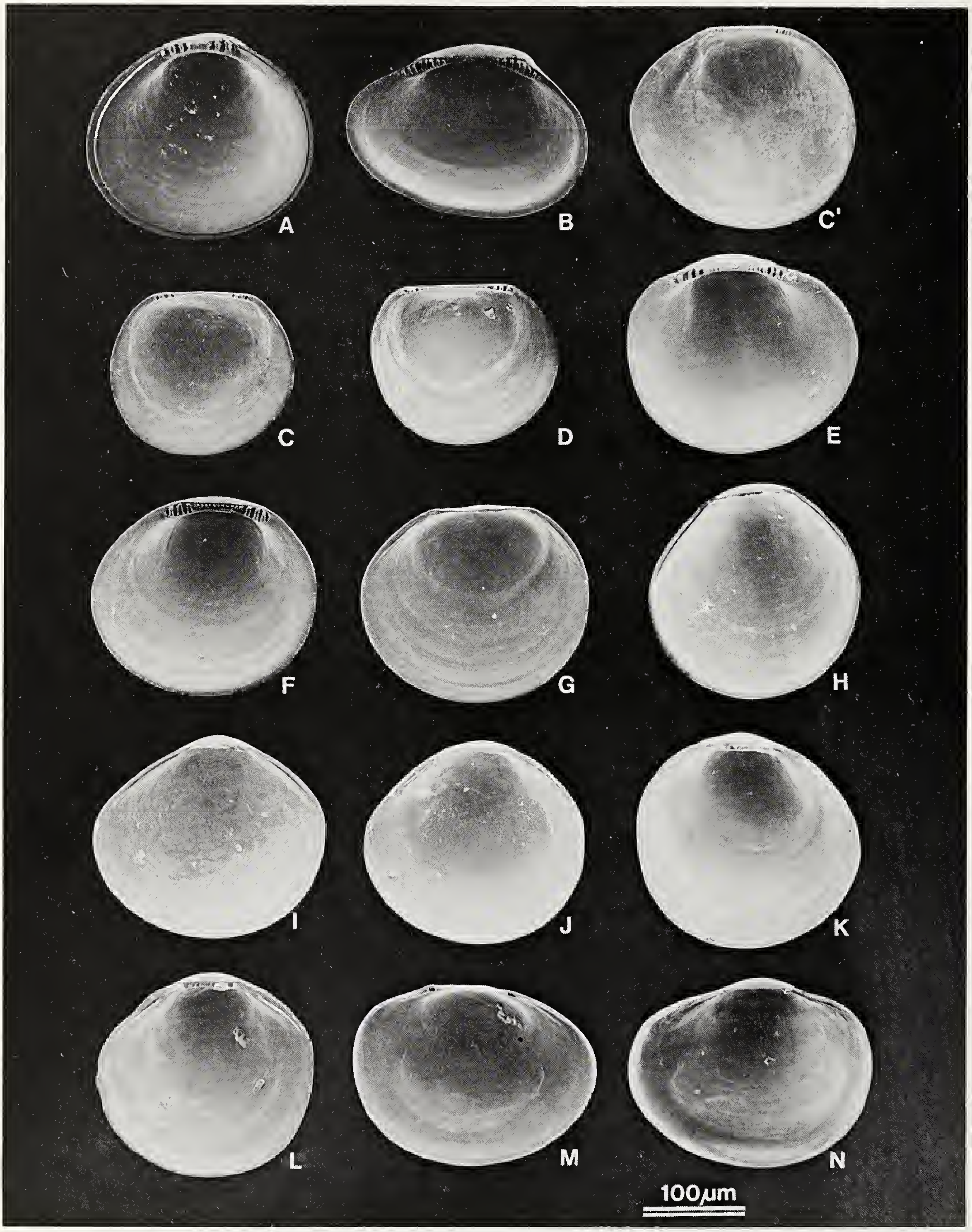


Fig. 16. Scanning electron micrographs of disarticulated shell valves of planktonic larvae of various species of bivalve molluscs. (A) *Crassostrea virginica* (Gmelin, 1791) (right valve, mature larva). (B) *Arca noae* (right valve; mature larva). (C') *Argopecten irradians* (Lamarck, 1819) (right valve; mature larva). (C) *Argopecten irradians* (left valve; straight-hinge larva). (D) *Placopecten magellanicus* (Gmelin, 1791) (left valve; straight-hinge larva). (E) *Mytilus californianus* Conrad, 1837 (left valve; mature larva). (F) *Geukensia demissa* (right valve; mature larva). (G) *Arctica islandica* (right valve; mature larva). (H) *Mercenaria mercenaria* (Linné, 1758) (right valve; mature larva). (I) *Mya arenaria* Linné, 1758 (right valve; mature larva). (J) *Mulinia lateralis* (Say, 1822) (right valve; mature larva). (K) *Spisula solidissima* (Dillwyn, 1817) (left valve, mature larva). (L) *Spisula solidissima* (right valve; mature larva). (M) *Ensis directus* Conrad, 1843 (left valve; mature larva). (N) *Ensis directus* (right valve; mature larva). Reprinted with permission from Lutz *et al.* (1982b).

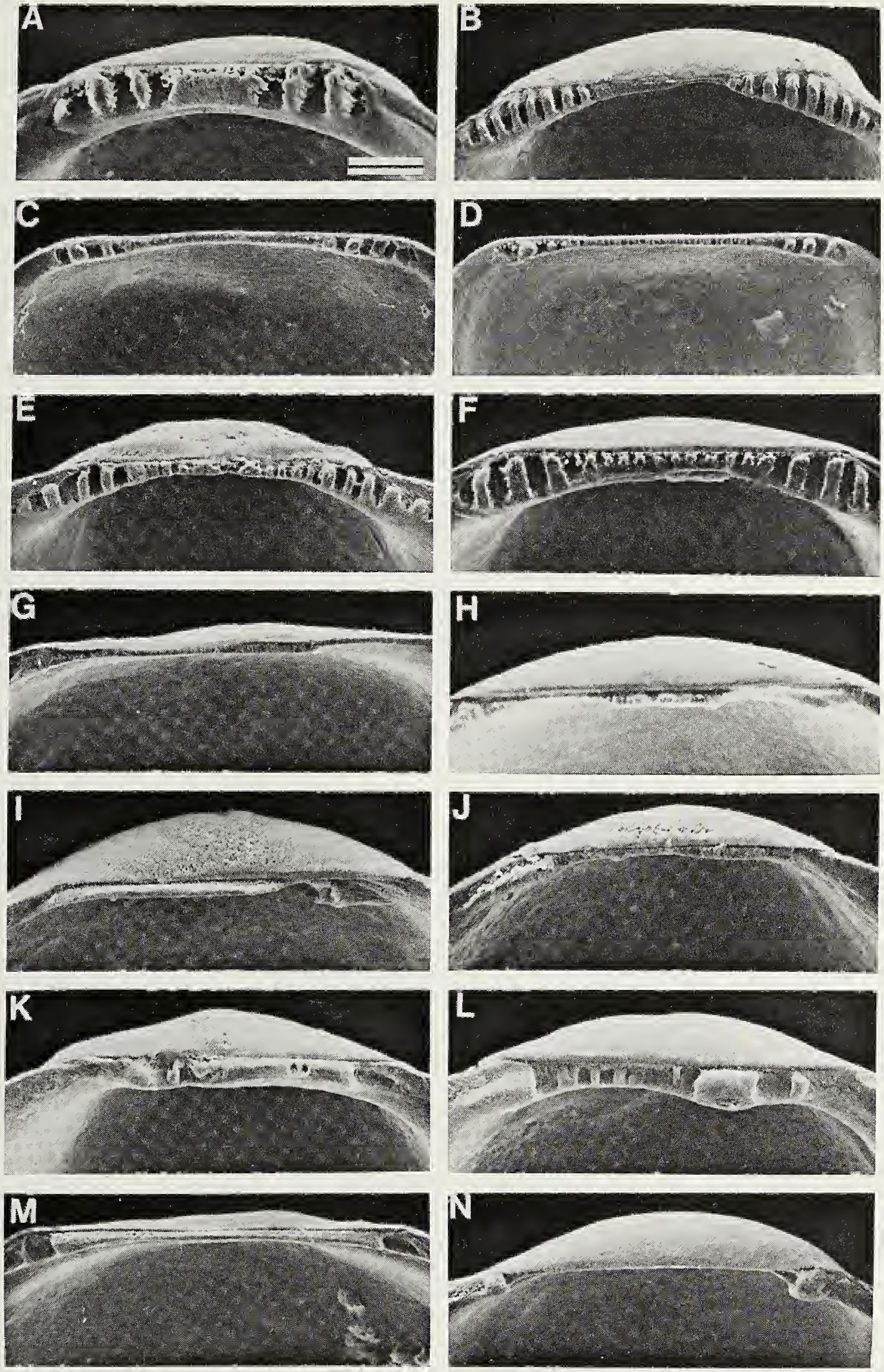


Fig. 17. Scanning electron micrographs of the hinge region of the disarticulated shell valves of the specimens depicted in Figure 16. (A) *Crassostrea virginica* (right valve). (B) *Arca noae* (right valve). (C) *Argopecten irradians* (left valve; straight-hinge stage). (D) *Placopecten magellanicus* (left valve; straight-hinge stage). (E) *Mytilus californianus* (left valve). (F) *Geukensia demissa* (right valve). (G) *Arctica islandica* (right valve). (H) *Mercenaria mercenaria* (right valve). (I) *Mya arenaria* (right valve). (J) *Mulinia lateralis* (right valve). (K) *Spisula solidissima* (left valve). (L) *Spisula solidissima* (right valve). (M) *Ensis directus* (left valve). (N) *Ensis directus* (right valve). Scale bar (= 20 μm) in A is applicable to all micrographs in this figure. Reprinted with permission from Lutz *et al.* (1982b).

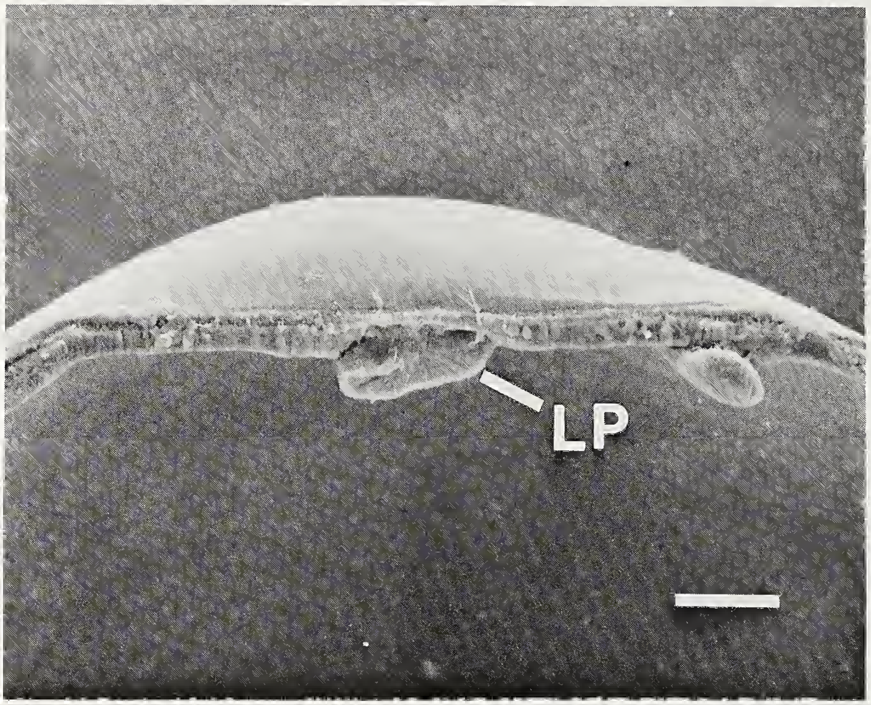


Fig. 18. Scanning electron micrograph of the hinge region of a disarticulated shell valve of *Macoma balthica* (Linné, 1758) depicting the primary ligament pit (LP). Scale, 10 μm.

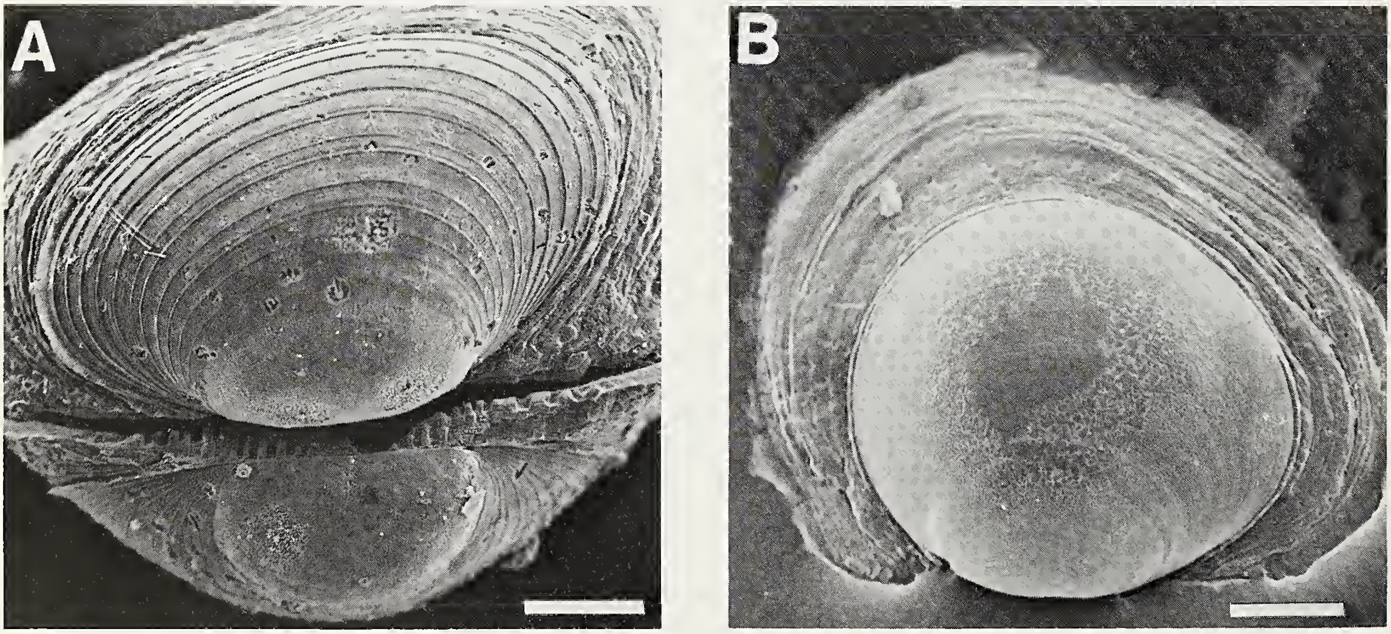


Fig. 19. Scanning electron micrograph of the umbonal region of early postlarval bivalves depicting the external shell morphology of the prodissoconch I and II and early dissoconch shells. (A) *Arca noae.* (B) *Argopecten irradians.* Scale, 50 μm.

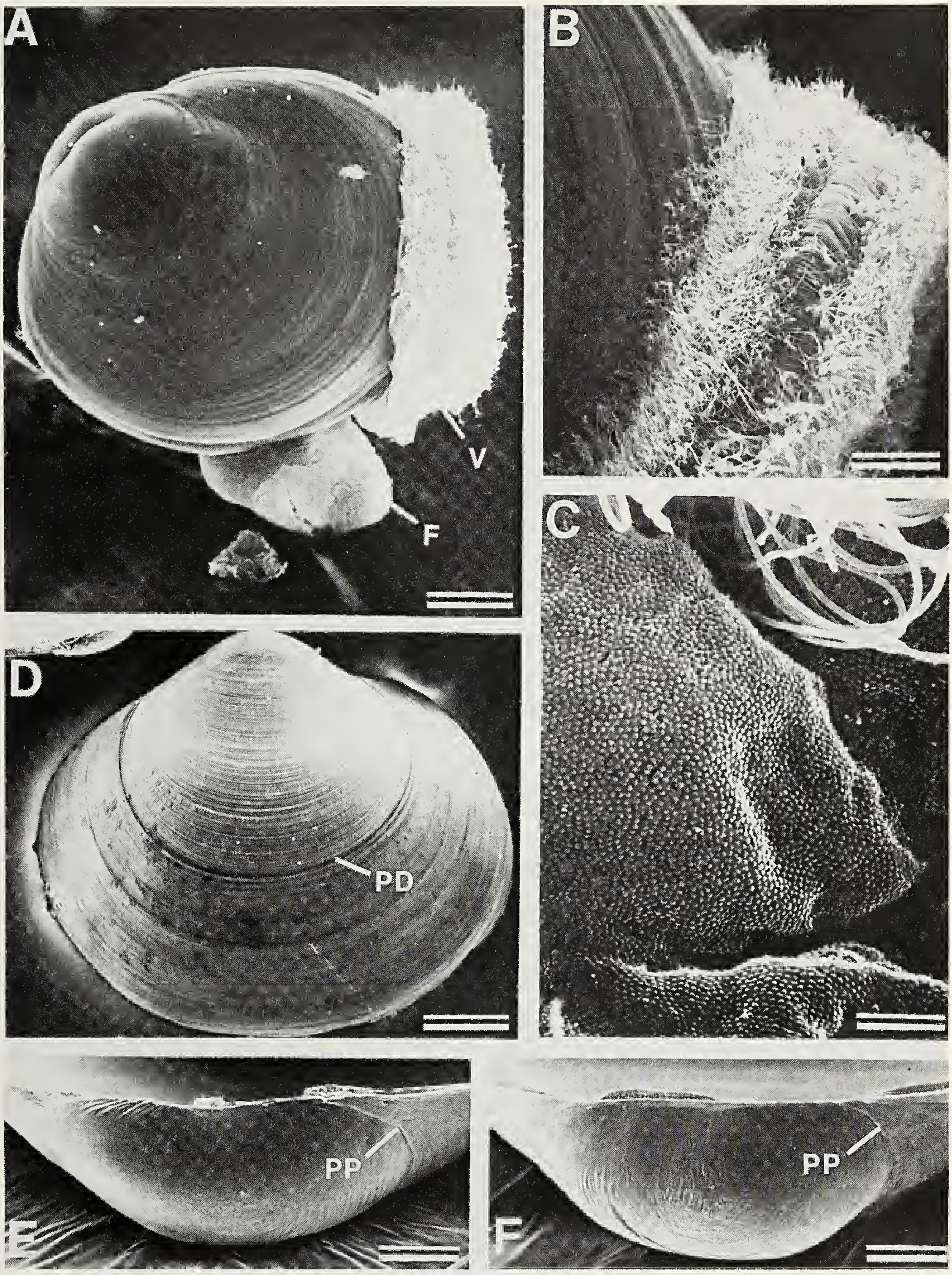


Fig. 20. Scanning electron micrographs of *Arctica islandica*. (A) Critical-point-dried pediveliger larva with extended velum (V) and foot (F). Scale, 50 μm. (B) Higher magnification of velar region of specimen seen in A. Scale, 20 μm. (C) High magnification of velum surface (anterior view) in region of apical pit (lower right) of specimen seen in A. Note cilia (top) of inner pre-oral band and microvilli over entire velar surface. Scale, 5 μm. (D) Disarticulated right shell valve of an early postlarva. Note distinct prodissoconch I-II boundary (PD). Scale, 75 μm. (E) Dorsal view of disarticulated right shell valve of a larva with a shell length of 232 μm. Note distinct prodissoconch I-II boundary (PP). Scale, 25 μm. (F) Dorsal view of disarticulated left shell valve of a larva with a shell length of 227 μm. Note distinct prodissoconch I-II boundary (PP). Scale, 25 μm.

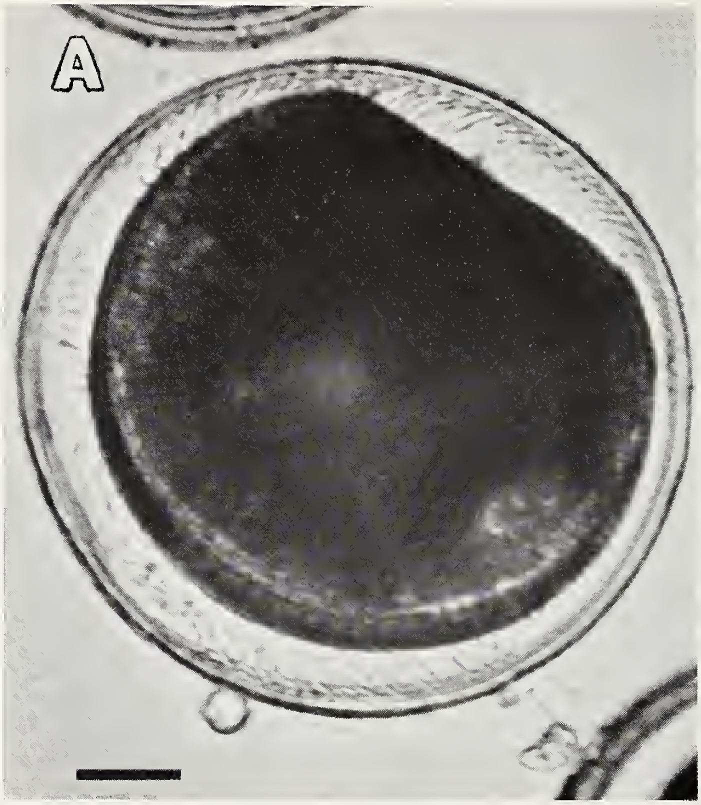
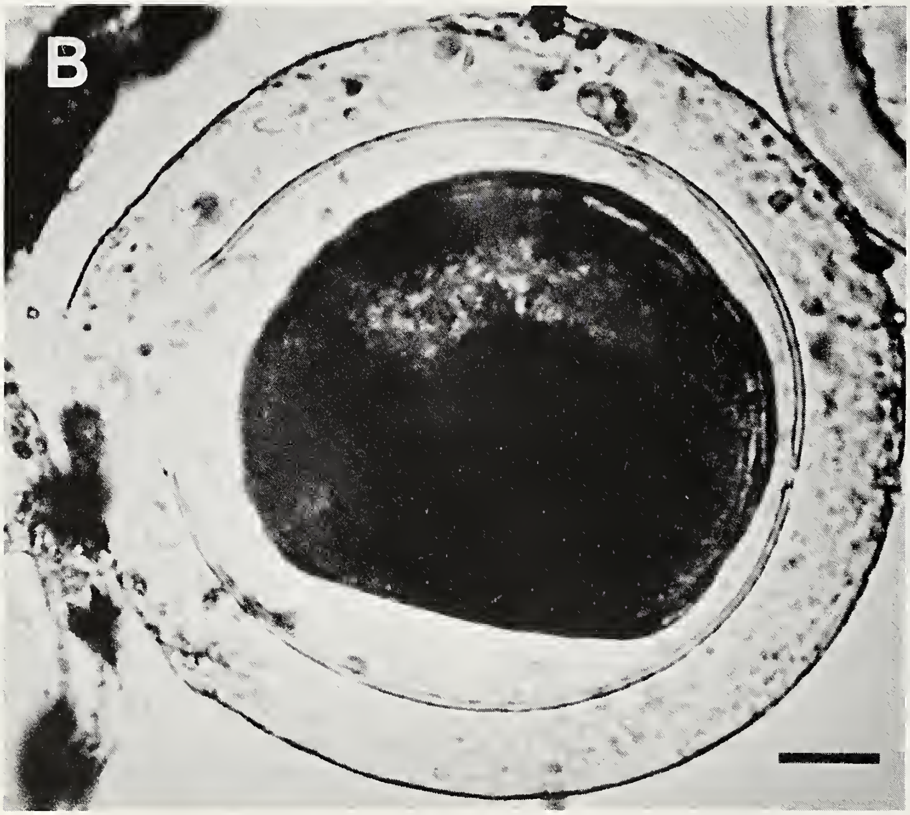


Fig. 21. Encapsulated stages of two continental shelf bivalves. (A) *Cyclocardia borealis* (Conrad, 1831). (B) *Astarte castanea* (Say, 1822). Development in both species is "direct", with the encapsulated stage lasting approximately 3-4 weeks in *Astarte* (at 14°C) and over 100 days in *Cyclocardia* (at 10-14°C). Scale in both micrographs, 50 μm.



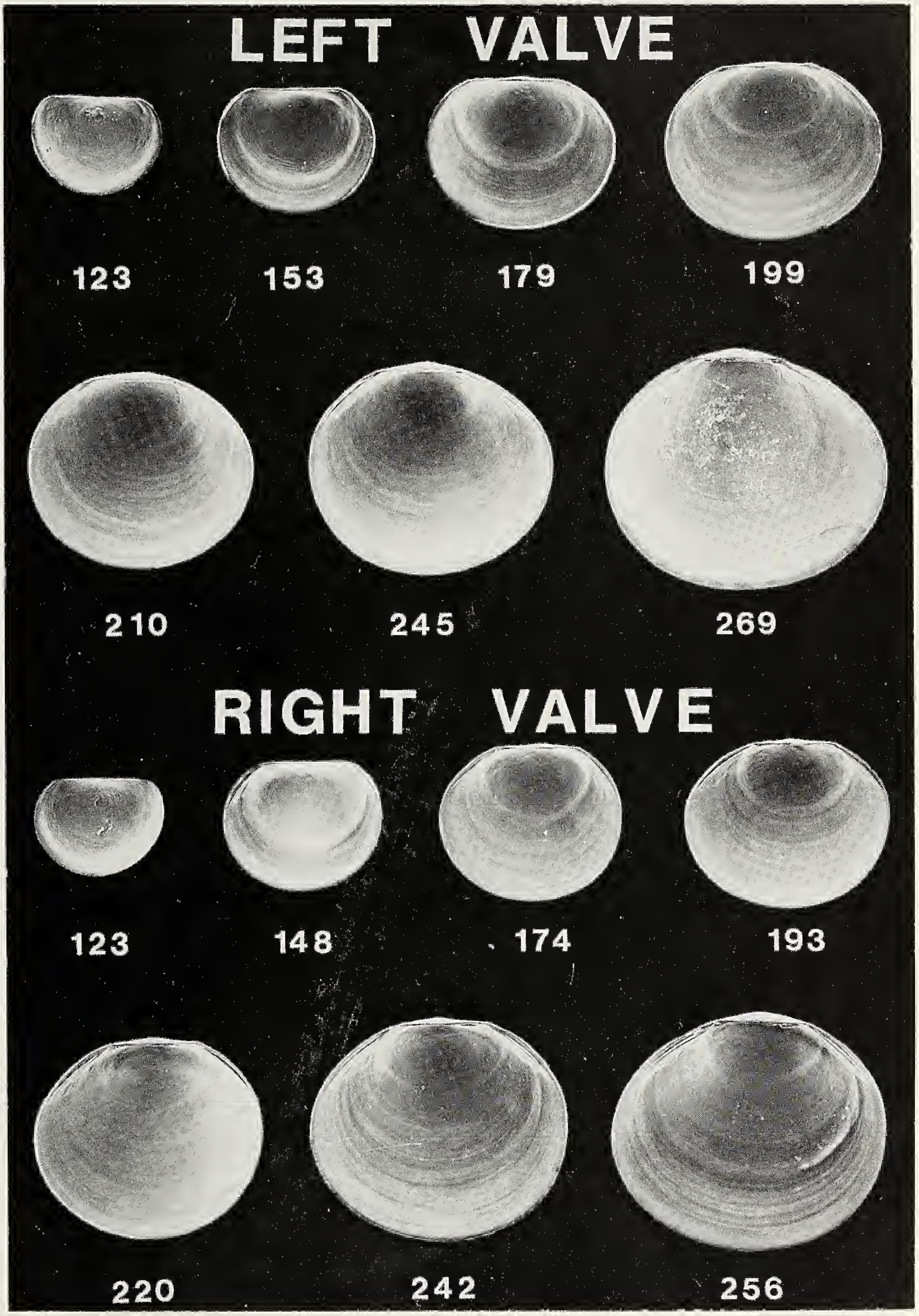


Fig. 22. Scanning electron micrographs of disarticulated shell valves of *Arctica islandica* larvae from early straight-hinge stage to metamorphosis. Numbers designate length of the shell (μm). Reprinted with permission from Lutz *et al.* (1982a).

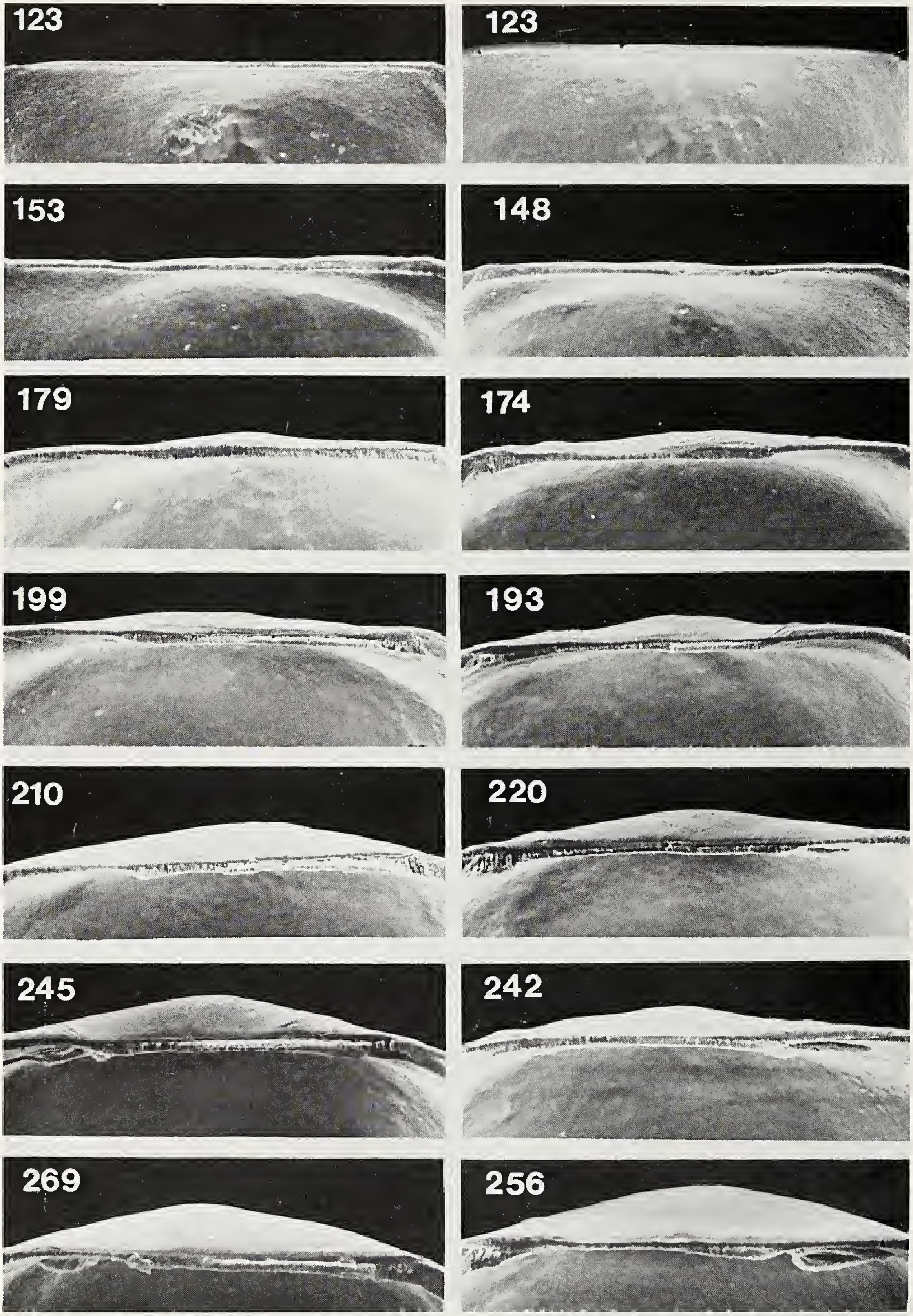


Fig. 23. Scanning electron micrographs of the hinge region of the disarticulated shell valves of *Arctica islandica* larvae seen in Figure 22. Left valves on left side and right valves on right side. Numbers designate length of the shell (μm). Reprinted with permission from Lutz *et al.* (1982a).

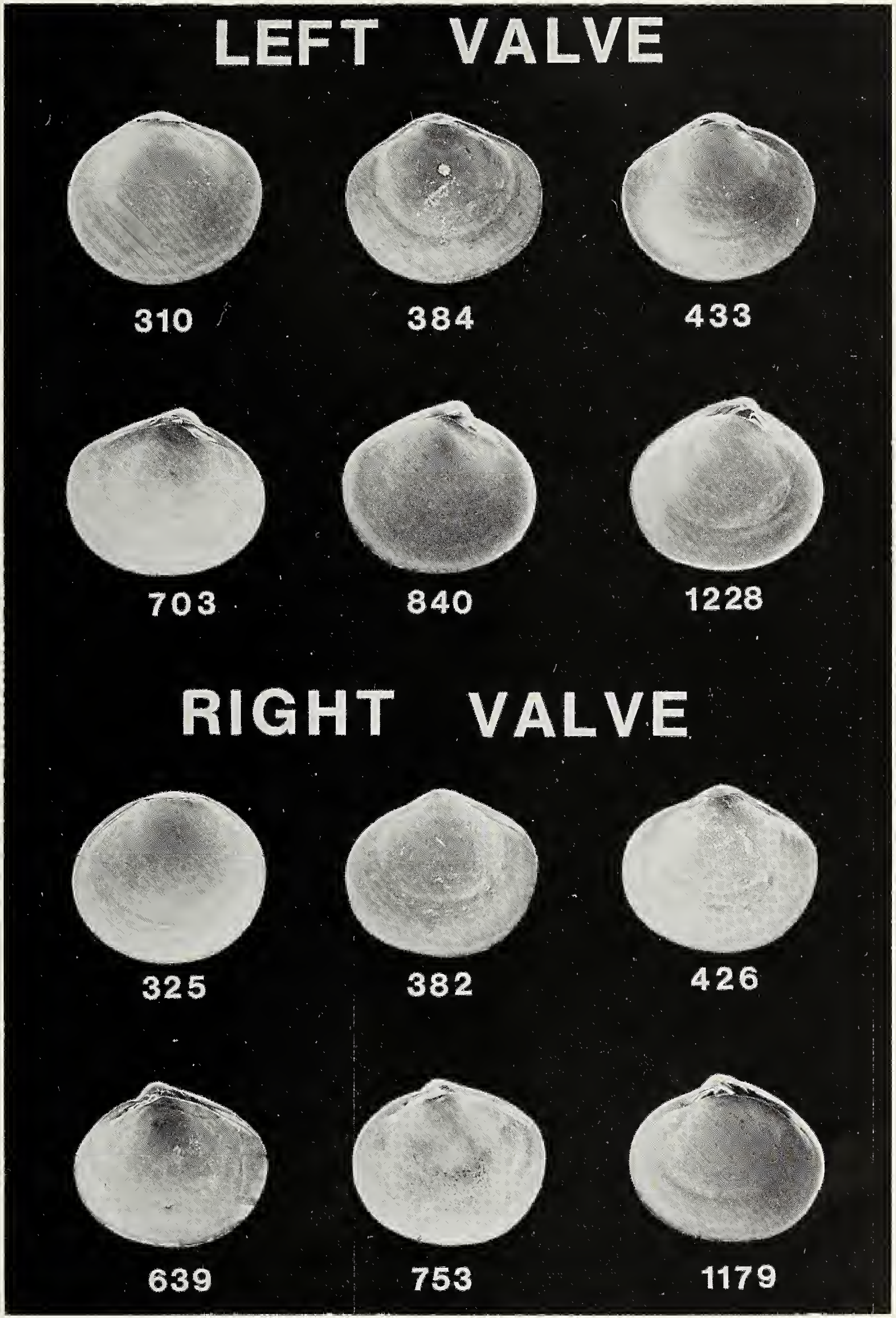


Fig. 24. Scanning electron micrographs of disarticulated shell valves of *Arctica islandica* postlarvae. Numbers designate length of shell (μm). Reprinted with permission from Lutz *et al.* (1982a).

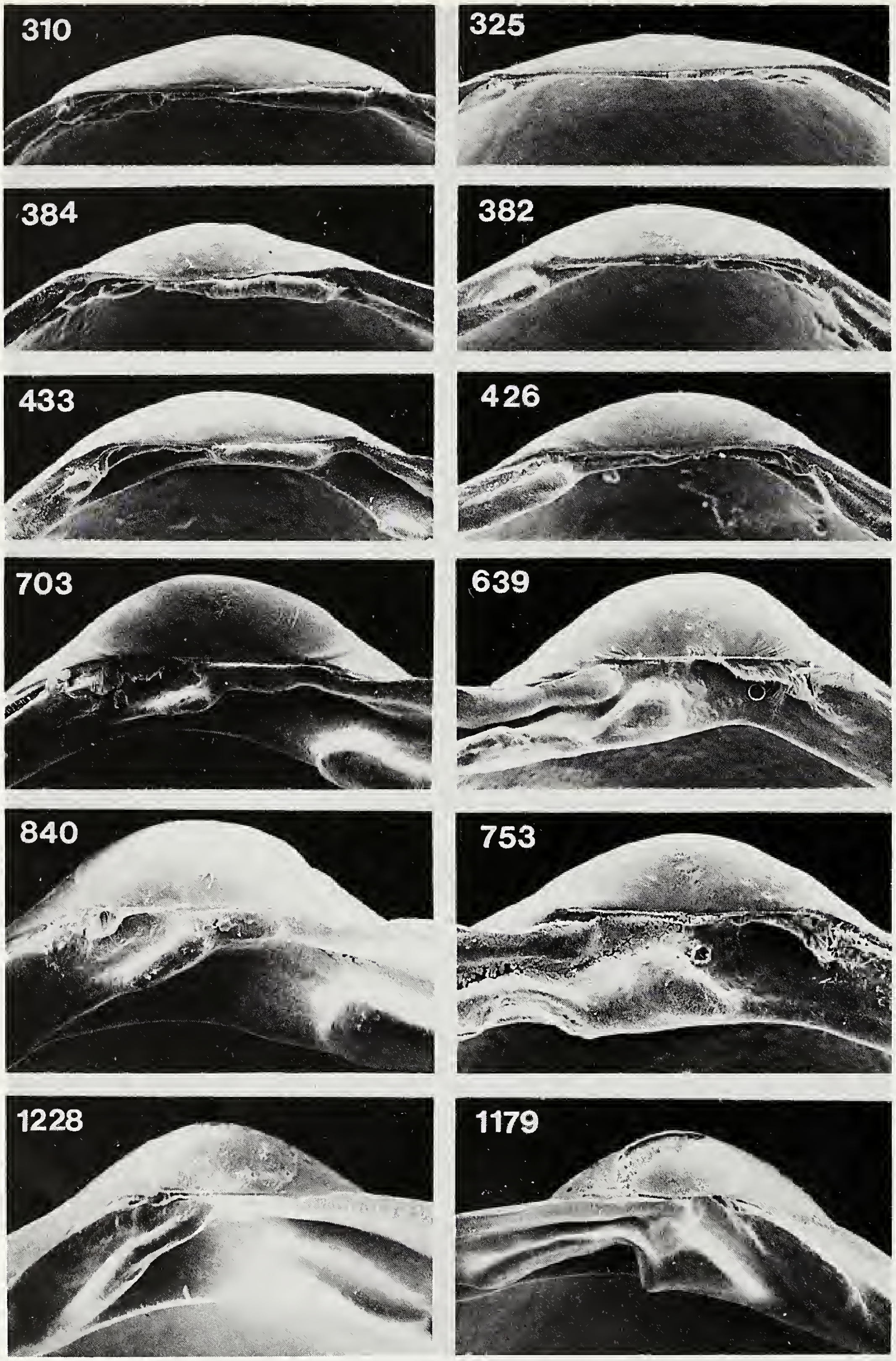


Fig. 25. Scanning electron micrographs of the hinge region of the disarticulated shell valves of *Arctica islandica* postlarvae seen in Figure 24. Left valves on left side and right valves on right side. Numbers designate lengths of the shell (μm). Reprinted with permission from Lutz *et al.* (1982a).

RECOGNITION AND REACTION OF MOLLUSCS TO FOREIGN SUBSTANCES

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ABSTRACT

The ability to recognize and react to foreign material is a fundamental biological property of cells. Cell mediated recognition of both self and nonself is the basis for all immune systems. The immune systems of vertebrates and invertebrates are here compared and contrasted and areas of research concerning molluscan immune systems are highlighted. It is concluded that, while it is possible to infer that molluscs do recognize and react to foreign substances in their tissues, it is not yet possible to state exactly how they do it.

The ability to recognize and react to foreign material is one of the most fundamental biological properties of cells. This phenomenon has been recognized in animals ranging from protozoa to mammals. In single-celled organisms these reactions are interpreted as feeding wherein protozoa identify nonself material, ingest it, and digest it to obtain needed nutrients. In multicellular organisms there are a variety of mechanisms that identify and neutralize foreign material and they are called immune systems. Their purpose is thought to be prevention or modulation of infection that would lead to tissue damage, disease, and/or death of the organism.

Cell mediated recognition of both self and nonself is the basis of all of these systems. Current major research efforts are focused on the molecular basis of recognition and cell-cell interactions. Vertebrate systems are complex and involve many cell types with specialized functions that are only now beginning to be understood in detail. Invertebrate systems have not been as thoroughly studied but they seem to lack this high order of cellular complexity. The information available currently indicates that invertebrate and vertebrate systems are, in general, dissimilar. This essay will compare briefly the principles of vertebrate and invertebrate immune systems, cellular recognition mechanisms, and explore areas of research to which molluscan studies can contribute.

GENERAL PRINCIPLES OF VERTEBRATE IMMUNE SYSTEMS

1. NONSPECIFIC MECHANISMS

Epithelial surfaces exclude most potentially harmful substances. Skin and mucus membranes present both mechanical and chemical barriers that exclude and/or destroy infectious agents and other foreign materials.

The mechanical component is the multilayered epithelium and the products secreted onto epithelial layers (e.g., organic acids, lysozyme, etc.) are the chemical components. Together these barriers exclude most of the potential invaders in the environment. Beneath these surface layers are various cells specialized for endocytosis, i.e., the uptake and destruction of foreign particles and solutes. Phagocytes are arrayed in the vertebrate body as either motile or fixed cells. Motile ones, such as polymorphonuclear leukocytes and monocytes, are in the circulation and during the process of inflammation they are mobilized rapidly at infection sites. Fixed cells (e.g., macrophages), are abundant in such tissues as liver, spleen, bone marrow, and lymph nodes. These tissues and cells are strategically arranged along routes of blood flow so that foreign substances in the systemic circulation are readily phagocytosed.

2. SPECIFIC MECHANISMS

Phagocytosis and processing of foreign material subsequently triggers specific reactions by populations of lymphocytes. Lymphocytes are derived from primitive stem cells and when mature several functional subtypes can be identified. "B" cells produce specific immunoglobulins of several classes. "T" cells interact with B cells to promote (T_H-helper cells) or supress (T_S-suppressor cells) immunoglobulin production. Other lymphocytes are conditioned to react with antigens directly or after coating with immunoglobulins. Lymphocytes under specific antigenic stimulation release soluble materials (lymphokines) that signal other cells to perform specific functions such as nonmigration, division, or secretion. This cell-to-cell communication greatly enhances the organism's ability to mount complex reactions to external or internal threats by establishing networks of cooperative defensive cells.

Cellular communication is based on common sequences of events in affected cells. First, a specific molecule in the host cell membrane combines chemically with a specific ligand (binding site) characteristic of the foreign material. Second, the foreign material and receptor are cleared from the surface and internalized. Third, the cell is stimulated to perform a metabolic function often resulting in the release of a cell product. The mechanisms responsible for that first critical recognition step are currently of great research interest.

GENERAL PRINCIPLES OF MOLLUSCAN IMMUNE RESPONSES

Epithelial surfaces of molluscs exclude foreign material as they do in vertebrates. Mechanical barriers, such as the shell of molluscs, and chemical barriers, such as molluscan mucus, undoubtedly represent substantial protection from foreign substances. These mechanisms certainly inhibit tissue penetration by a very large number of potentially deleterious substances.

Foreign material that does penetrate molluscan tissue is met by motile cells called hemocytes. They are active phagocytes and have some macrophage-like properties. They digest or export foreign material quite effectively and help to maintain tissue integrity (Tripp, 1963). In addition, there are soluble substances in the hemolymph that react selectively with some foreign materials. They have some antibody-like characteristics but are clearly different from immunoglobulins. There is evidence that they may serve as opsonins (i.e., enhance phagocytosis) but that in other cases they are not required for phagocytosis. Bayne (1983) has listed the most important unanswered questions about mechanisms of recognition of foreignness by molluscs. Among them are: 1) what chemical characteristics of the molluscan hemocyte enable them to recognize foreign substances? and, 2) is there cell-to-cell communication in molluscs? These are not easily answered questions because there is no general agreement on the lineage of molluscan hemocytes and techniques for manipulating molluscan hemocytes are not as advanced as those used for vertebrate cells.

REACTIONS TO FOREIGN SUBSTANCES

The ability of cells to discriminate among different particle types is very widely distributed among animal phyla (Jenkin, 1976). For example, the protozoan *Amoeba proteus* will preferentially ingest the flagellate *Chilomonas* or the ciliate *Colpidium* in preference to the flagellate *Monas* (Mast and Hahnert, 1935). In metazoa, phagocytes play important roles in defense, nutrition, development and differentiation (Jenkin, 1976). The original observations by Metchnikoff of *Daphnia* infected with fungus being protected by phagocytes gave rise to current concepts of the special importance of these cells (Metchnikoff, 1905). There is limited evidence that invertebrates can mount true immunological reactions with demonstrable memory. In the cockroach (*Periplaneta americana*) a secondary response has been demonstrated (Karp and Rheins, 1980). Annelids (Cooper, 1975), echinoderms (Karp and Hildemann, 1975), and corals (Hildemann *et al.*, 1975) show transplantation immunity.

The functions of molluscan hemocytes have been reviewed recently by Bayne (1983) and their discriminatory abilities summarized by Chorney and Cheng (1980). Briefly, molluscan phagocytes react to the presence of many kinds of nonself materials including a variety of infectious agents as well as inert and living materials introduced experimentally. Also, self material that is damaged or aged so as to be nonfunctional is removed as if it were nonself. There are some exceptions, however. The snail *Biomphalaria glabrata* reacts to the presence of some strains of *Schistosoma mansoni* but not others (Richards, 1976). Similarly, the same strain of *S. mansoni* will successfully infect some strains of *B. glabrata* but not others (Richards, 1975).

Clearly molluscs can identify foreign substances and react to them (Chorney and Cheng, 1980). Different reactions can be seen depending on the stimulus, but circulating hemocytes are always involved. Phagocytosis of foreign substances is followed by digestion (Tripp, 1958) or exportation to the exterior (Tripp, 1960). Larger parasites are encapsulated to a greater or lesser extent depending on whether the mollusc host-parasite is a compatible combination or not (Yoshino, 1976). Immune recognition of the parasite seems to require specific receptors on the snail hemocyte (Bayne *et al.*, 1984). Actual killing of the parasite seems to depend on inherent cytotoxic properties of snail hemocytes (Bayne *et al.*, 1980; Loker and Bayne, 1982). Quite fine distinctions may be made among foreign molecules by molluscan hemocytes. For example, hemocytes of the chiton *Liolophura gaimardi* remove different hemocyanins from the hemolymph at different rates; the most foreign molecule is removed fastest (Crichton and Lafferty, 1975).

It seems clear that molluscs make fine distinctions among foreign substances in their tissues. These reactions are mediated by hemocytes. Two types of mechanisms may be postulated: 1) hemocytes have specific receptor molecules on their membranes that allow identification of foreignness, or 2) some other mechanism marks a foreign substance in some way that stimulates a subsequent reaction by hemocytes.

MOLLUSCAN HUMORAL FACTORS

Numerous soluble substances in the hemolymph of many molluscs have been described (Tripp, 1966, 1975;

Michelson and Dubois, 1977; Bayne, 1983) and their role in defense mechanisms has been reviewed recently by Fries (1984). In general these substances are large (100-1000 Kd) proteins or glycoproteins (Boswell and Bayne, 1984). The primary structure of these substances is not like immunoglobulin (Acton *et al.*, 1969), although they often have antibody-like specificity for red blood cells, bacteria, or parasites. The role these humoral factors play in resistance is still unclear (Chorney and Cheng, 1980). The suggestion has been made, however, that lectins attach to foreign substances and subsequently signal "foreign" to hemocytes. This cannot always be true, however, since molluscan hemocytes phagocytose particles *in vitro* in medium free of hemolymph; when hemolymph is added, phagocytosis may be enhanced (Tripp, 1966). Thus lectins are not essential for phagocytosis but may improve its efficiency. This apparant anomaly can be explained by a model that depicts the lectin attached to the hemocyte membrane. Support for this idea comes from recent work by Vasta *et al.* (1984) with oyster *(Crassostrea virginica)* hemocytes. These workers found that the membrane component was similar to the soluble hemolymph lectin but thought it unlikely that it was merely the soluble form adsorbed onto the hemocyte surface. Renwrantz and Stahmer (1983) found a similar serological relationship between *Mytilus edulis* hemocytes and lectins.

Although molluscan lectins are widely distributed and relatively well studied, it still is not clear what their function is or how they may function in recognition of foreignness. This may be clarified by definitive experiments soon, but in the meantime it is useful to turn to studies on the nature of hemocyte membranes.

MOLLUSCAN HEMOCYTES

There is not universal agreement on the lineage of molluscan hemocytes or the nature of their mature forms (Jeong *et al.*, 1983; Joky, *et al.*, 1983; Sminia *et al.*, 1983) although a functional classification has been proposed recently by Cheng (1984). However, there is evidence that within circulating hemocyte populations the macrophage-like granulocytes are subpopulations that differ in cell surface characteristics (Yoshino and Granath, 1983). These differences have been found in both bivalves (*C. virginica*) (Yoshino *et al.*, 1979; Vasta *et al.*, 1984) and gastropods (Yoshino and Granath, 1983). Early evidence was indirect in that there was variability of hemocyte affinity for various test particles (e.g., red blood cells, bacteria; Tripp, 1975). More precise information has become available recently due to surface membrane analysis with monoclonal antibodies (Yoshino, 1983a). The molecules identified thus far fall into four classes: 1) glycoproteins reactive with lectins of known specificity (e.g. Concanavalin A); 2) hemolymph-like proteins; 3) fibronectin-like membrane components; and 4) determinants similar to the murine Thy-1 antigen (Yoshino, 1983b). Apparently these different molecules are spread randomly over the surface of the cells, and, when activated by specific lectins, patching and capping result (Yoshino, 1982). Also there is evidence that some surface antigens may be linked (Yoshino and Davis, 1983). These observations are strikingly similar to those made on certain vertebrate cells involved in immune reactions.

As cell surfaces are characterized biochemically it is becoming clear that all hemocytes are not equal. *Biomphalaria glabrata* hemocytes treated with monoclonal antibodies can be divided into two distinct subpopulations (Yoshino and Granath, 1983). Oyster (*C. virginica*) hemocytes may also consist of subpopulations defined by their surface receptors (Yoshino *et al.*, 1979; Vasta *et al.*, 1984). It still remains to be demonstrated definitely whether these differences are because immature cells differ from mature ones or whether there are distinctly different subpopulations of mature hemocytes.

These recent findings suggest strongly that molluscan hemocytes are complex structurally and that subpopulations may exist. This is consistent with other lines of biochemical evidence showing variation in quantities of lysosomal enzymes in *Mya arenaria* (Huffman and Tripp, 1982) and *B. glabrata* (Granath and Yoshino, 1984). The relationship between hemocyte membrane structure and function remains unclear, but it is reasonable to presume that cells with different membrane components have different recognition capabilities.

These differences raise other interesting possibilities about cell-to-cell interactions in molluscs. If there are subpopulations of cells with different capabilities, is it possible that they may communicate via chemical signals? A possible example of activation of hemocytes from *S. mansoni*-susceptible snails *(B. glabrata)* by humoral factors from resistant strains of the same snail species has been reported by Bayne *et al.* (1980). This phenomenon is well established as an essential part of vertebrate immune reactions that greatly enhances the effectiveness of the cellular response system. The possibility that chemical signals between molluscan hemocytes can lead to cooperative action deserves careful attention. Even rudimentary communication would cause a drastic change in thinking about the roles these cells may play and their importance in maintaining the integrity of molluscs.

DISCUSSION AND CONCLUSIONS

While it is possible to infer that molluscs do recognize and react to foreign substances in their tissues, it still is not possible to say precisely how they do it. Vertebrates use a sophisticated dual coding system to recognize nonself material and the lymphocytes that are activated release chemical signals that stimulate other effector cells. It is very unlikely that such a complex system will be found in molluscs but some elements could be present. Recently published information suggests that the molluscan cell membrane presents a more complicated landscape that anyone imagined heretofore. The cellular source and functions of lectins is unclear but they clearly are associated with hemocytes and can distinguish among a great variety of biological particles. The possibility of subsets of hemocytes having different capabilities as end cells seems reasonable and the ability of some selected cells issuing chemical commands to others is not out of the question.

Finally, molluscan cells are not always easy to work with for technical reasons but they may offer uniquely useful systems for studying cell-cell interactions. It will be interesting to see how this promising area of research develops in the next few years.

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DELAYED METAMORPHOSIS OF MARINE MOLLUSCAN LARVAE: CURRENT STATUS AND DIRECTIONS FOR FUTURE RESEARCH

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ABSTRACT

The larvae of many marine invertebrates are capable of delaying their metamorphosis beyond the time that they first become capable of metamorphosing to adult form and habitat. The ability to delay metamorphosis increases the probability of post-metamorphic survival, and increases dispersal potential during larval life, thus possibly influencing degree of genetic exchange among adult populations, rates of speciation, and species longevity. This paper reviews what is presently known about delayed metamorphosis in marine molluscs, and suggests additional lines of research: 1) determining maximum duration of the delay period and total length of larval life for larvae reared under controlled conditions in the laboratory, 2) documenting the influence of changes in environmental conditions on delay periods and total length of larval life, 3) determining the conditions under which metamorphosis is delayed in the field, 4) understanding the physiological and genetic basis for the onset of metamorphic competence and for spontaneous metamorphosis or death, 5) determining the costs to fitness, if any, resulting from delaying metamorphosis, and 6) determining the degree to which differentiation proceeds during delayed metamorphosis. Such studies will increase our understanding of the ecological significance of delayed metamorphosis, and of the mechanisms through which selection can bring about different delay periods among species and among individuals within species.

Many benthic marine molluscs have a free-living larval stage in the life history (Thorson, 1946; Hardy, 1965). These larvae must go through a period of development in the plankton before becoming capable of metamorphosing to the benthos. During this so-called precompetent period of development, the larvae can not be induced to metamorphose (e.g., Crisp, 1974; Bonar, 1978; Chia, 1978; Hadfield, 1978) and there is thus an obligate period of dispersal in the life history of these otherwise sedentary species. At some point in development, the larvae become competent to metamorphose; that is, they become capable of responding to specific physical and chemical cues in the environment by metamorphosing (Crisp, 1974; Gray, 1974). These cues are typically associated with some aspect of the adult environment (e.g., Gray, 1974; Switzer-Dunlap, 1978; Morse *et al.*, 1979; Burke, 1983). This ability of competent larvae to choose where they will eventually metamorphose is clearly adaptive in that, by metamorphosing into environments particularly appropriate for the juveniles and adults of the species, the probability of surviving to reproductive maturity is increased (Thorson, 1950; Crisp, 1974).

In a developmental context, it is often difficult to define metamorphosis in a useful way. Certainly, metamorphosis involves rapid morphological and often physiological differentiation, but differentiation occurs both prior to and following metamorphosis, and the extent of differentiation occurring at metamorphosis varies considerably among taxa. In an ecological context, however, the point in development at which metamorphosis occurs is more readily defined. Among the bivalves, gastropods, chitons, and scaphopods, the larvae are planktonic whereas the adults are benthic, so that metamorphosis involves a distinct and very pronounced ecological transition. In practice, then, molluscan metamorphosis is typically defined by the loss of the locomotory organs or locomotory cilia (e.g., Bonar and Hadfield, 1974; Hadfield, 1978; McFadien-Carter, 1979; Pechenik, 1980, 1984), which effectively ends the period of planktonic dispersal in most species.

Among the bivalves, gastropods and scaphopods, metamorphosis involves the irreversible loss of the velum, a ciliated, uniquely larval organ used for food collection, gas exchange, and swimming in the plankton. Polyplacophorans (chitons) do not exhibit a veliger stage in their development, but do pass through a free-swimming, ciliated trochophore stage (Pearse, 1979); chiton metamorphosis involves the irreversible loss of the locomotory cilia (Barnes and Gonor, 1973; Watanabe and Cox, 1975; Pechenik, 1984a).

Most research conducted on molluscan metamorphosis to date has focused on describing morphological changes that occur during metamorphosis (e.g., Bayne, 1971; Hickman and Gruffydd, 1971; Bonar, 1976, 1978; Bonar and Hadfield, 1974; Bickell *et al.*, 1981; Bickell and Kempf, 1983), or on identifying environmental cues that trigger metamorphosis in particular species, determining the active components (chemical and physical) of these cues, and attempting to understand how stimuli are perceived by larvae and translated into the ecological, physiological and morphological revolution that often constitutes metamorphosis (e.g., DeBlock and Geelen, 1958; Perron and Turner, 1977; Hadfield, 1978; Morse *et al.*, 1979; Heslinga, 1981; Morse and Morse, 1984; Baloun and Morse, 1984; older literature reviewed by Crisp, 1974; Gray, 1974; Scheltema, 1974).

However, few studies have looked specifically at what happens to larvae if the appropriate cues are never encountered. In the absence of cues, metamorphosis can be delayed (Thorson, 1950; Crisp, 1974; Gray, 1974). Maximum dispersal potential of a species, or individual, thus depends on the duration of the precompetent period of development and the amount of time that metamorphosis can subsequently be delayed (Pechenik, 1984). The ability of molluscan larvae to delay metamorphosis is what I wish to discuss in this paper.

The question of delay capability is a question worth addressing for a number of reasons, at least some of which are ecological. The ability to delay metamorphosis can apparently at least double the maximum duration of larval life (Scheltema, 1971; Pechenik, 1980; Jackson and Strathmann, 1981), and thus is expected to play an important role in determining the extent of genetic exchange between populations, in determining rates of speciation, and in determining geological longevity of species (e.g., Shuto, 1974; Scheltema, 1978; Hansen, 1978; Strathmann, 1978, 1980; Jablonski and Lutz, 1983; Valentine and Jablonski, 1983). Nevertheless, delay periods are poorly defined and poorly understood for marine molluscs.

Based on the limited research that has been conducted to date, the following statements can be made:

1. Growth and differentiation may continue during the delay period. It had been thought that once larvae became competent to metamorphose, all further development ceased (Scheltema, 1966, 1971), but for the larvae of the four prosobranch species that have been studied in some detail in the laboratory, growth continues long after larvae become capable of metamorphosis (Pechenik, 1980, 1984b; Lima and Pechenik, in press). Differentiation may also take place during the period of delayed metamorphosis; in particular, gills, which generally appear following metamorphosis, develop during the delay period (Pechenik, 1980; Pechenik and Lima, 1984). Although opisthobranch larvae are known to cease growth during the time of delayed metamorphosis, morphological differentiation continues in some species after cessation of shell growth (Bickell and Chia, 1979) and physiological differentiation clearly continues as well, as the larvae do not become competent to metamorphose until several days after growth has stopped (Kriegstein, 1977; Switzer-Dunlap and Hadfield, 1977; Kempf, 1981).

Long-distance, teleplanic larvae (Scheltema, 1971) may provide another exception to continued growth during the delay of metamorphosis. These larvae are believed capable of delaying metamorphosis for many months, long enough to permit transoceanic larval transport (Scheltema, 1971). Measurements made on preserved teleplanic larvae of the prosobranch gastropod *Cymatium parthenopeum* (von Salis) show no indication of increases or decreases in shell length or biomass as the larvae drift across the Atlantic Ocean (Pechenik *et al.*, 1984). It is not known whether this apparent cessation of growth is innate, or simply a result of low open-ocean food levels. Whether or not differentiation ceases during delayed metamorphosis and whether larvae collected in mid-ocean retain the capacity for metamorphosis have not yet been determined. Studies of larval physiology for teleplanic species are only now being initiated.

2. Metamorphosis can apparently not be delayed indefinitely, even by plankton-feeding larvae. For the few species reared in the laboratory under controlled conditions and without deliberately triggering metamorphosis, the larvae either eventually metamorphose spontaneously, or they die (e.g., Bayne, 1965; Chia, 1978; Pechenik, 1980, 1984a,b; Jackson and Strathmann, 1981). The eventual fate of chiton larvae, *Mopalia muscosa* (Gould), reared in glass dishes (cleaned daily) in the laboratory seems to vary with temperature regime (Pechenik, 1984a), but even so, the length of larval life was always finite.

3. Species, and individuals within a species, seem to differ substantially in the length of time required to become competent for metamorphosis, and in the length of time that metamorphosis can subsequently be delayed (Hadfield, 1977; Pechenik, 1980; Jackson and Strathmann, 1981; Pechenik and Lima, 1984). More studies in which researchers set out specifically to define delay periods for particular species under controlled conditions in the laboratory are required before generalizations about variability in potential length of larval life can be made.

The adaptive significance of differences in ability to delay metamorphosis are unclear, as are the pressures selecting for such differences within and among species. Presumably, species with more specific, patchily distributed habitats will have greater delay capabilities than those with more general, frequently encountered habitats (Crisp, 1974; Obrebski, 1979; Pechenik, 1980; Bickell and Kempf, 1983). To test this hypothesis, we need to accumulate more data on delay capabilities of larvae reared in the laboratory. We also need field data on adult habitat availability for a variety of species and on how habitat availability changes over time. In addition, we need data on how often and under what conditions the larvae of different species actually delay metamorphosis in the field. Although the phenomenon of delayed metamorphosis has been demonstrated to occur in the field for the larvae of several mollusc species (the bivalve *Mytilus edulis* (Linné) — Bayne, 1965; the gastropods *Bittium alternatum* (Say), *Crepidula fornicata* (Linné) and *C. plana* (Say) — Pechenik, 1978), the field conditions under which delayed metamorphosis occurs have not been explored.

We also need to know more about how changes in environmental conditions (salinity, temperature, food quantity, food quality, presence of environmental pollutants) affect delay periods. With such information, we may become better at predicting delay periods from knowledge of other, more readily measured aspects of development, and better at predicting how delay periods will change with changes in environmental conditions.

We also need to understand not only the ecological basis, but also the physiological and developmental basis for differences in delay capabilities among species. By understanding the mechanisms behind such differences, we may discover the vehicles through which natural selection can operate to select for different delay periods in different species. For lecithotrophic larvae, which subsist on yolk and do not feed on particulates in the water column, selection for maximum dispersal period may operate through selection for rate of energy expenditure and amount of yolk allocated to individual embryos (Lucas *et al.*, 1979; Pechenik *et al.*, 1979; Pechenik, 1984a.) If lecithotrophic larvae are capable of taking up and metabolizing dissolved organic material, as demonstrated for the planktotrophic larvae of several mollusc species (Rice *et al.*, 1980; Langdon, 1983; Manahan, 1983), the role of yolk supply in limiting length of larval life might be lessened. Nutritional limitation seems unlikely to play a major role in determining maximum length of larval life for planktotrophic species that never lose the ability to feed in the plankton.

Some of these questions about the role of internal and external factors in determining the potential duration of delayed metamorphosis have recently been pursued using the larvae of the prosobranch gastropods *C. fornicata* and *C. plana* (Pechenik, 1980, 1984b; Pechenik and Lima, 1984; Lima and Pechenik, in press), in part because larvae of these species show excellent survivorship in culture (generally in excess of 90%), so that little of the genetic variability present at the beginning of an experiment is lost during the study. In particular, there is very little mortality during the delay period of either species; virtually every individual eventually exhibits spontaneous metamorphosis when reared in clean glass containers.

In each experiment, larvae are obtained from a single hatch and distributed among a number of different treatment groups. In some experiments, the larvae have been reared at different temperatures, in the presence of excess phytoplankton (Pechenik, 1984b; Pechenik and Lima, 1984; Lima and Pechenik, in press). In general, it was found that larvae that grew rapidly reached the point of spontaneous metamorphosis sooner than those that grew more slowly. That is, there was a distinct, inverse relationship between rates of growth and length of larval life prior to spontaneous metamorphosis. This relationship was observed for larvae growing at different rates at a single temperature, and for larvae reared at different temperatures. At any given temperature, a distinct range of maximum dispersal periods was associated with a given growth rate.

Larvae of the mussel *Mytilus edulis* also show the shortest delay periods when reared at temperatures (and salinities) producing the highest pre-competent growth rates (Bayne, 1965). However, the results obtained for mussel larvae by Bayne differ from results obtained for *Crepidula fornicata* and *C. plana* in that delaying larvae of *Mytilus edulis* don't metamorphose spontaneously; instead, most of the larvae undergo pronounced morphological degeneration and eventually die (Bayne, 1965). Specifically, the velum and mouth region degenerate, and the larvae consequently become incapable of feeding. These degenerative events occur sooner when larvae are reared at temperatures and salinities producing rapid rates of growth (Bayne, 1965).

Additional species must be studied before any generalizations can be made. However, the data for *Mytilus edulis, Crepidula fornicata* and *C. plana* suggest that there is a pre-determined, developmentally programmed end to larval life (Chia, 1978; Pechenik, 1980, 1984b), so that the maximum possible length of larval life depends upon the rate at which individuals differentiate towards this larval endpoint (Pechenik, 1984b). The existence of a pre-determined end to larval life would provide a simple vehicle through which natural selection could bring about different delay periods in different species and among individuals within one species; this could be achieved, at least in part, through selection for different rates of development (Pechenik, 1984b).

According to this hypothesis, the reason that we see a distinct inverse relationship between growth rate and maximum length of larval life is presumably because a change in temperature alters rates of growth and rates of differentiation in approximate proportion to each other; elevating temperature increases not only growth rate, but also the rate of progress towards the larval endpoint. If rates of growth and differentiation could be uncoupled, this inverse relationship should disappear.

This inverse relationship between rate of growth and length of larval life is observed even at constant temperatures, when developmental rate is varied by altering food concentration, as indicated in an experiment for *C. plana* reared on a diet of *Isochrysis* sp. (Tahitian isolate) at 25°C (Fig. 1). In general, larvae grew faster at the higher food levels and exhibited earlier spontaneous metamorphosis, as also seen in the temperature experiments. However, some indication of uncoupling between rate of growth and length of larval life appears to occur above growth rates of approximately 60 μm/day. Above this point, further increases in rates of growth are not accompanied by further decreases in length of larval life. Even so, there was no significant difference in the size at

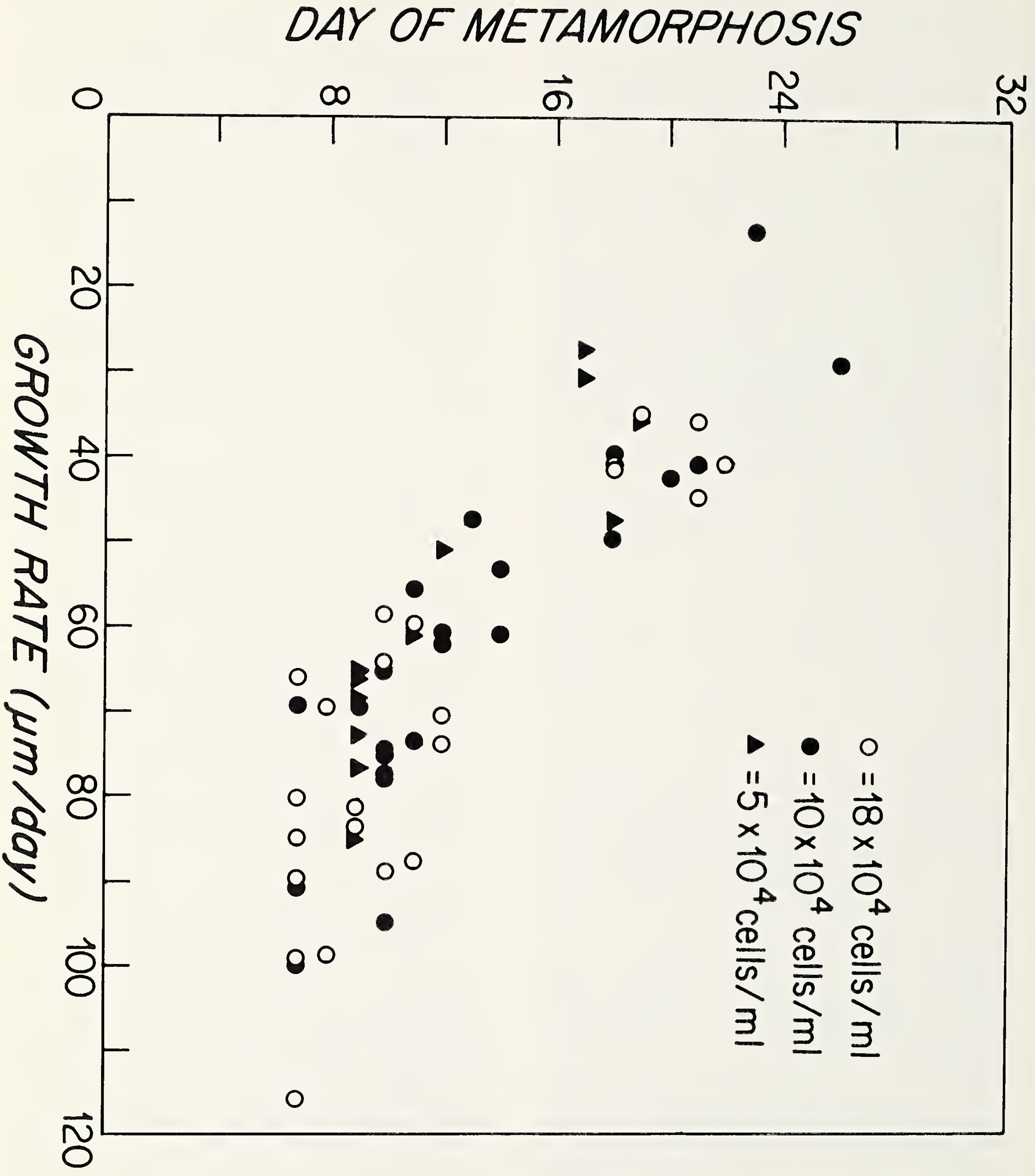

Fig. 1. Duration of larval life as a function of estimated individual growth rate for *C. plana* reared at 25°C on three different concentrations of the naked flagellate *Isochrysis* sp. (Tahitian isolate).

metamorphosis for larvae in the different food treatments.

Results to date have been intriguingly different when larvae are reared under the above conditions, but on different food concentrations of the local isolate, *Isochrysis galbana* (Parke). Although larvae grew faster at higher food concentrations in these experiments, length of larval life did not correlate with rate of growth. Moreover, spontaneous metamorphosis occurred at significantly smaller sizes when larvae were reared on the most dilute diet, and at significantly larger sizes when larvae were reared on the most concentrated diet. These results must be treated cautiously, since experiments using the two diets were completed in different years; further studies are now being initiated. However, the present results suggest that by altering the diet, rates of growth and rates of differentiation have been uncoupled. For example, increasing the food concentration in this second set of experiments increased growth rates but apparently had little effect on the rate of differentiation towards the hypothesized larval endpoint, so that metamorphosis occurred at a large shell length.

The apparent ability of a change in diet to uncouple rates of growth and length of larval life is interesting from a practical viewpoint. In aquaculture systems, a major goal is to maximize productivity by promoting high larval survivorship and rapid development to metamorphosis. Food value is generally assessed in terms of larval survivorship and growth rates (e.g., Davis and Guillard, 1958; Walne, 1963; Wilson, 1979; see review by Pechenik, in press). According to the above results, however, the diet that provides the fastest growth rate may not be the diet that provides the shortest length of larval life. The relationship between rates of growth and rates of differentiation requires further study. In particular, the conditions under which rates of growth and rates of morphological and physiological differentiation are uncoupled would seem to warrant further attention.

Delay of metamorphosis may also warrant attention at the molecular level. To what extent does differentiation proceed during the so-called delay of metamorphosis? To what extent is differentiation held in abeyance? Through what vehicle is differentiation suppressed? Larvae that delay metamorphosis may provide useful systems for studies into the control of gene expression. Usually, people studying the control of gene expression work with some dormant system, such as an egg, turn it on, and then study differentiation at the molecular level (Browder, 1984). With delayed metamorphosis, we have a system that is differentiating, and is primed to continue differentiating, but in the absence of a specific chemical or physical cue, does not differentiate, at least not to the fullest extent possible. Delay of metamorphosis may provide a viable alternative approach to understanding control of gene expression.

If delay of metamorphosis is to play major roles in maintaining genetic exchange between geographically separated adult populations and in the processes of speciation and extinction, juveniles derived from larvae that have delayed metamorphosis must be as fit as those derived from larvae that have not experienced a substantial delay period. If delaying metamorphosis decreases subsequent fitness, however, the ecological significance of delayed metamorphosis would be reduced.

Costs to fitness could take any of several forms: reduced probability of survival to sexual maturity (mediated through reduced resistance to physical and biological stress); delayed onset of sexual maturity; reduced fecundity; or reduced viability of offspring (e.g., Stearns, 1976; Berven, 1982). This possibility of reduced fitness has not yet been explored. However, there is no obvious reason why the competent period of a feeding larva should not extend until an appropriate habitat is encountered, assuming that larvae metamorphosing into such habitats have a higher probability of survival to maturity than those that metamorphose sooner into less appropriate habitats (Crisp, 1974; Pechenik, 1984a). The phenomenon of spontaneous metamorphosis in glass containers (cleaned daily) suggests that costs to fitness may well occur during delayed metamorphosis; if postponing metamorphosis beyond a certain time incurred such a cost to the larva (through, for example, reduced tolerance of environmental stresses or increased susceptibility to predation) or to the metamorphosed juvenile, selection would not act to prolong larval life indefinitely.

In summary, we know very little about the ability of molluscan larvae to delay metamorphosis. What we do know is intriguing, but there are many questions remaining to be pursued. These questions can be addressed through both laboratory and field investigations, and can be pursued from many perspectives: descriptive, ecological, physiological, developmental, and molecular. The questions seem to be interesting ones. I expect that the answers will be, too.

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IS THERE A SQUID IN YOUR FUTURE: PERSPECTIVES FOR NEW RESEARCH

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ABSTRACT

This paper presents information about future perspectives for research on cephalopods. The objectives and functions of the Cephalopod International Advisory Council (CIAC) are described as an introduction to recognized needs in cephalopod research. Through workshops and symposia CIAC intends to present current research and to stimulate and promote future research. Specifically addressed are new areas of research needed to advance systematic and evolutionary biology, whole-animal biology and fisheries biology. Suggestions stress the application of new techniques and new approaches to augment traditional studies in these fields.

Cephalopods never have been studied so intensely as they are at the present time. While research on cephalopods has shown a steady increase since the mid-1940s, just as in many other groups of organisms, it has increased very dramatically in the past 15-20 years. Why is this so? As basic research scientists, it is easy enough for us to present as a major reason the recognition of the significant role cephalopods play in many marine ecosystems, as they are conspicuous top-level predators as adults and are major prey at all life stages; an understanding of communities requires an understanding of their constituent parts. This has stimulated much-needed research on the biology of cephalopods. Also, we are aware of the extensive use of the squid giant axon in biomedical research; demands for increasing numbers and a year-round supply of animals for neurophysiological and other biomedical applications have stimulated much research on life histories and behavior, especially in relation to rearing and culturing. Less obvious to most North Americans, at least, is the importance of cephalopods as a human food resource. Now, increasing acceptance at home and an ever-increasing demand on the world market have seen the worldwide catch of cephalopods quadruple during the past few decades to around 1.5 million metric tons annually. The desirability to preserve currently exploited stocks on a maximum sustained yield basis, the concomittant necessity to develop new fisheries on underutilized species, and the development of new processing techniques have stimulated a significant amount of research on systematics, distribution, life history, ecology and population dynamics.

The most tangible evidence of the recent intensity of cephalopod research comes from the unprecedented number of comprehensive publications that have been published on a very broad range of topics in the past dozen years. Voss (1973) assessed for the Food and Agriculture Organization of the United Nations (FAO) the cephalopod stocks on a worldwide basis for the first time; this was followed by an updated assessment by Okutani (1977) based primarily on the preeminent Japanese cephalopod fisheries that dominate the worldwide catch statistics. A large volume of papers was published as a result of the symposium honoring Professor J. Z. Young, edited by Nixon and Messenger (1977). The series of papers presented at the 1980 American Malacological Union Symposium on "Functional Morphology of Cephalopods" was published in *Malacologia* (vol. 23, no. 1, 1982). The proceedings of the large international workshop on "The Biology and Resource Potential of Cephalopods" held in Australia in 1981 were published in book format (Roper, Lu, and Hochberg, Eds., 1983). In addition to the 26 research papers, the volume contains a summary of recommendations for future directions in cephalopod research. An international symposium on squid fisheries was held in Boston in 1981, with the proceedings appearing in 1982 (UNIPUB, 1982). The Food and Agriculture Organization (FAO) published an extensive volume assessing cephalopod resources (Caddy, 1983). A very important 2-volume series on life cycles in cephalopods was compiled by Boyle (1983, In press). Another FAO publication was the worldwide catalogue of cephalopods important to fisheries (Roper, Sweeney, and Nauen, 1984). Clark (In press)

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has compiled an important handbook on the identification of cephalopod beaks from the stomachs of predators. Results of species-specific or topic-specific meetings or symposia are published in Anon. (1980); Balch, et al. (1978); Granger (1983); Rowell (1981). Papers that resulted from the Cephalopod Expedition of the *Alpha Helix* are published in Arnold (1983). These recent major publications serve as examples of the expanding interest in research on the biological aspects and resource potential of cephalopods.

The recognition of the growing importance of cephalopods resulted in a burgeoning of research around the world. An increasing number of biologists were being encouraged or assigned to work on cephalopods, often without prior training or exposure to cephalopod biology or taxonomy and the relevant (often difficult to find) scholarly literature. Therefore, results often were variable; papers were published in a wide diversity of biological and fisheries publications, some of them quite difficult to obtain. Because of these circumstances, it became quite clear to a number of cephalopod specialists that a conspicuous lack of direction from the international viewpoint had developed, the continuation of which could lead to much inefficiency, redundancy and misdirection in the field. So, at the International Workshop on the Biology and Resource Potential of Cephalopods, held in Melbourne, Australia in March, 1981, the concept of establishing an international body was introduced and accepted. During the workshop on cephalopod beaks from predator stomachs held at the Marine Biological Laboratory, Plymouth, England in June 1981, the charter members convened a working group to develop the organizational structure for the Cephalopod International Advisory Council (CIAC). The Charter Committee then met at Laboratoire Arago, Banyul-sur-Mer, France, in September 1983 to formally accept the charter and resolutions and to elect the first regular council members.

The membership of CIAC consists of 18 internationally recognized scientists who specialize in research on cephalopods. Membership is on a rotating basis in order to maintain stability and direction and at the same time to provide opportunity for input from as broad a spectrum of cephalopod research interests and geographical representation as possible. The current council consists of members from Australia, Canada, England, France, Japan, South Africa, Spain, and the United States. These members represent the following fields of cephalopod expertise: systematics, zoogeography and evolutionary biology; comparative and functional morphology, embryology, developmental biology and life histories; parasitology; ecology; behavior; mariculture; and fisheries biology and resource development.

The objectives of CIAC are: 1) to stimulate and to influence scholarly research on cephalopods from an international perspective; 2) to provide information, advice, and assistance on all aspects of cephalopod biology, including those associated with the development and management of cephalopod fisheries resources; and 3) to disseminate information on past and current cephalopod research. To achieve these objectives CIAC will monitor and review the current status of research on cephalopods on a worldwide basis to provide an overview of results, directions, trends, needs, and problems. This will be accomplished through organization and sponsorship of international workshops, symposia, and training courses; production of handbooks, computerized, cross-indexed bibliographies, "state of the art" papers and a periodic newsletter on living cephalopods (i.e., Recent, not fossil); maintenance of a list of experts available as consultants to any individual, organization, or government requiring information, advice, and assistance on any aspect of cephalopod biology and fisheries. CIAC is especially interested in working with scientists, students, and organizations of developing countries, both in terms of delineating their cephalopod fauna taxonomically and biologically and of determining the potential for developing these cephalopod resources for domestic consumption and for foreign exchange.

CIAC has become affiliated with the International Council of Scientific Union's International Association of Biological Oceanography (IABO), sponsored through UNESCO. Further, the CIAC charter provides for a representative of the Food and Agriculture Organization of the United Nations to serve as a permanent observer on the Council.

The preceding introduction provides the background for the comments that follow. If these suggestions seem tied closely to the CIAC objectives it is because of my close association with the conception, establishment, and functioning of that organization and of my conviction that CIAC will provide the international direction and stability needed for future development of cephalopod research. CIAC and its precursor, the Workshop on the Biology and Resource Potential of Cephalopods, have addressed the topic of future research needs and directions. This is the very subject of our symposium dedicated to Mel Carriker, and since the CIAC organization, objectives, and plans have not yet been introduced to the malacological community at large, I take this opportunity to do so and to develop themes currently under discussion in CIAC. In doing so, I draw from and acknowledge the following sources: the introduction and papers published in the Proceedings of the Workshop on Biology and Resource Potential of Cephalopods (Roper, Lu, and Hochberg, Eds., 1983); and, especially, personal communications with Roger T. Hanlon, Marine Biomedical Institute, Galveston, Texas; F. G. Hochberg, Santa Barbara Museum of Natural History, Santa Barbara, California; and Warren Rathjen, formerly National Marine Fisheries Service, Gloucester, Massachusetts; currently Florida Institute of Technology, Melbourne, Florida. I am most grateful for the discussions with these three colleagues that contributed in a major way to the development of this paper. I am very grateful to Prof. T. Okutani, Tokyo University of Fisheries, who reviewed the paper and provided many helpful suggestions. I also acknow-

ledge with gratitude the helpful comments of two anonymous reviewers.

THE FUTURE

The establishment of CIAC by working scientists as a recognized international organization has enabled the rapid implementation of objectives. For example, as a result of a written survey of many cephalopod scientists from around the world, CIAC concluded that the single most urgent problem facing cephalopod researchers is the inability to identify "larval" and early juvenile stages of cephalopods. Here was a clearly recognized need to which CIAC was able to respond by organizing and sponsoring an international workshop, followed by a larger symposium on the Biology and Distribution of Early Juvenile Cephalopods, held in June, 1985, at Laboratoire Arago, Banyul-sur-Mer, France. Since the beginning of studies on cephalopods, the identification of virtually all stages of young and juvenile forms has been a confusing and problematic endeavor; the inability to identify the young stages has created a serious impediment to progress of research in cephalopod ecology, systematics, and fisheries. This is especially the case for neritic and oceanic species, those that constitute the huge biomass upon which the major fisheries are based, including the numerous species of *Loligo*, the adults of which provide the giant nerve axons used in biomedical research. In recognition of the urgent need for new techniques of identification, scientists from a variety of disciplines participated in the workshop, e.g., comparative morphology, biochemistry, systematics, and behavior of living "larvae", participated in the workshop.

The specific aims of the workshop were to 1) develop and refine standardized methods of identifying larval and early juvenile growth stages of cephalopods to the species level; 2) report current research on various aspects of biology of early growth stages of cephalopods, especially concerning identification, distribution, abundance, ecology, behavior, and prey-predator relationships; 3) formulate future research directions based upon a worldwide view of cephalopod resources available for basic and applied research; 4) discuss future workshops and collaborative programs based upon needs and views of the workshop participants and 5) publish a handbook resulting from the findings of the larval workshop and a special volume in "Vie et Milieu" of the papers presented at the symposium.

The 12-day Banyuls workshop followed the format established during the successful workshop held in Plymouth, England in June, 1981 on cephalopod beaks from predator stomachs, results of which are published in a handbook format (Clarke, In press). Thirty-five cephalopod researchers from around the world participated in the Banyuls workshop, with an additional 20 attending the symposium.

In attempting to address future perspectives in cephalopod research, I find it convenient to subdivide the field into basic components. While this serves the purposes for discussion, we recognize that most research entails a mixture of these components.

Systematics and Evolutionary Biology. Estimates of the number of living species of cephalopods vary from about 500 to perhaps 1,000, depending upon the number of undescribed species one wishes to incude. My guess is about 1,000. The rate of discovery of species new to science remains high, especially in geographical areas and inaccessible habitats previously little studied. While the description of new species certainly serves an important function, the real challenge that continues to face systematists is to produce revisionary and up-to-date monographic works, including functional keys to identification, on genera and families based upon comprehensive collections and utilizing modern systematic techniques. The status of systematic knowledge of most cephalopods is relatively primative and this situation creates impediments to progress in research in many other fields of cephalopod biology, e.g., ecology, behavior, and fisheries. If an ecologist or fishery biologist is unable to distinguish biological species with certainty, the research results or resource management plans surely will be flawed.

While some very fine systematic work has been done on cephalopods (see reviews in Voss, 1977; Roper, 1983), certainly fewer than 100 species are well understood from the systematic standpoint. The excellent volume entitled "Cephalopod Life Cycles" edited by Peter Boyle (1983) demonstrates my point. The 23 species discussed are among the best known biologically of all cephalopods. Yet, 17 of these species belong in the four families designated by an international workshop of cephalopod experts as being in critical need of systematic revision on a world-wide basis: Sepiidae, Loliginidae, Ommastrephidae, Octopodidae (see Introduction in Roper, Lu, and Hochberg, Eds., 1983). Of the remaining six species, only *Nautilus macromphalus* Sowerby, 1848 is in a taxonomically stable family.

Illex illecebrosus (Lesueur, 1821), an ommastrephid, is the major commercial species of squid in the western North Atlantic, where annual catches in Canadian and U.S. waters now occasionally exceed 100,000 metric tons. Because of the commerical importance of this species, much research has been done on its biology and population dynamics (see O'Dor, 1983), but several important factors remain unknown, e.g., mating and spawning sites and early life history of hatchlings and juveniles in relation to distribution, water masses, etc. Systematic problems still exist in spite of the large amount of knowledge gained in the past decade. *Illex illecebrosus* seems to be the only species of *Illex* north of the Mid-Atlantic Bight, but southward to Florida *I. oxygonius* Roper, Lu, and Mangold, 1969 occurs sympatrically. These two species also occur in the Gulf of Mexico where they are joined by *I. coindetii* (Verany, 1837), an amphi-Atlantic species. Adult members within the genus *Illex* in the northern Atlantic are easy to identify; in the area of sympatry, only the adult males of *I. illecebrosus* and *I.*

oxygonius are easily identified, while the females and immature individuals are nearly indistinguishable. Matters are worse in the Gulf of Mexico, where unreconcilable forms coexist with the three species. Larval specimens within the genus *Illex* are relatively easily distinguished from other ommastrephid larvae, but species identifications are still impossible. Further, western North Atlantic spawning sites for the three species are unknown; the larvae are "pelagic" at hatching and apparently are quickly intermixed and dispersed by oceanic currents. All fisheries catches of *Illex* are listed as *I. illecebrosus* and that species designation is applied to most of the research subjects as well. Because of the systematic problems, fisheries biologists are greatly hampered in their objectives to develop the fishery and their ability to make meaningful predictive judgments necessary for the development of rational utilization and management plans. Until the systematics are thoroughly understood, knowledge of all other aspects of *Illex* biology and resource utilization will remain incomplete.

Octopus vulgaris Lamarck, 1798 certainly is the most ubiquitous and extensively studied cephalopod in the world (Mangold, 1983). It is considered a world-wide species in tropical, subtropical and temperate waters and its identification is certain in the Mediterranean and eastern Atlantic where it is the only, or one of a very few, species of *Octopus*. Elsewhere, *O. vulgaris* is the name of choice applied to almost any nondescript medium-sized muscular octopus, whether it occurs alone or with a number of congeners. It is extremely important in fisheries as well as being the premier research cephalopod. Enough uncertainities exist about identification of *O. vulgaris* from less-studied areas, that it is risky automatically to apply biological knowledge acquired for the Mediterranean species to the supposed conspecifics in Southeast Asian waters, for example. On the other hand, to describe a new *O. vulgaris*-like species from an unstudied area can lead to just as many problems.

The point of these examples certainly is not to malign cephalopod systematists, but rather to demonstrate the very great need to encourage and support intensified systematic studies.

Future perspectives can be very encouraging for solving many systematic problems, especially through a combination of approaches. Of course, morphology remains the cornerstone of systematics; there simply is no substitute for fine, detailed morphological studies to show phylogenetic relationships. However, future systematic studies will benefit from increased attention to other aspects of cephalopod biology. Certainly zoologists and paleontologists can work more closely to gain a better understanding of the evolution and phylogenetic relationships of extant cephalopods, as well as an impression of the biology of forms long extinct. The work of Peter Ward and his colleagues is an example (e.g., Ward, 1979a and b). New analytical techniques will enhance the collaboration between paleontologists and zoologists. The best example of this is the x-ray photographic technique pioneered and developed by W. Stuermer (1970), by which delicately fossilized soft parts of animals can be detected in the slate matrix. Heretofore, only shells, beaks, and gladii were available in the fossil record of cephalopods. Now with the ability to "see" other significant anatomical features of systematic value, the door is open for major progress in cephalopod evolutionary studies. To that end, Professor Stuermer and I began collaborative studies in the summer of 1985.

Biochemical analyses, such as techniques for electrophoresis and mitochondrial DNA studies that are being conducted on other molluscan groups, should be applied to cephalopods as well. These could prove to be valuable tools for cephalopod systematists in conjunction with more traditional approaches.

Any future systematic work must recognize the great need to explore morphological features that may prove to be useful as systematic characters. Objective analytical techniques, such as must be applied, as was so well demonstrated in the paper by Voss and Voss (1983) that utilized cladistics. But systematists now should be prepared to go beyond working only with preserved specimens. A wealth of information applicable to systematics, as well as to other biological interests, can be acquired by observing living animals where their natural habitats are accessible to observers or when they can be maintained in the laboratory. For example, cephalopods are capable of producing extensive color changes, body patterns, and skin sculpturing that reflect specific behavioral responses to predators, mates, the environment, etc. (Packard and Hochberg, 1977). Observations are needed on whole-animal behavior: how color, patterns and sculpture are used; how prey are located, stalked, and captured; how mates are selected and mating proceeds; in short, how the animal makes its living in the real world—what are the living characteristics that distinguish it from its closest relatives? The living animals observed in the field will provide hints and ideas that will allow systematists to go back to the laboratory to study well-preserved specimens and to look for the detailed morphological differences. Eric Hochberg (pers. comm.) experienced a vivid example of this in Japan when two specimens of octopus were brought into the lab and kept alive; they were immediately identifiable as separate species based upon their skin patterning, even though preserved specimens seemed indistinguishable because the patterns disappear. Subsequent morphological study revealed consistent and characteristic differences, but no name could be assigned because the character had not been examined in that area before.

Because systematic informaton is so urgently required and because an animal's behavior, ecology, and habitat ultimately reflect a species-specific individuality in concert with morphological uniqueness, systematic biologists are challenged to undertake innovative approaches to solving age-old systematic problems.

Biology. An exciting future is seen for biological research on cephalopods. Recognition of the critical role cephalopods play in various marine communities as upper-level predators and as prey to apex predators, such as fishes, marine mammals and birds, and other large cephalopods, insures that they must receive future research attention. Cephalopods generally are highly mobile, short-lived, fast-growing, large-sized and highly fecund predators. Their distribution, habitat, feeding habits and behavior all can change with sequential growth stages. These and other generalities are well established in the literature. Now we need to accumulate the solid details to hang on the skeleton of existing knowledge.

Behavior may be at the central core for understanding most other aspects of cephalopod biology, e.g., seasonal and vertical migration; prey-predator interrelationships and strategies; reproductive biology; fisheries potential. The behavioral repertoire of cephalopods compared to that of other mollusks is staggering, and comparisons with highly evolved marine fishes show important parallels (Packard, 1966, 1972). Reflect for a moment on a pelagic squid versus an oyster; concurrent with the enormous morphological changes required for a soft-bodied animal that has abandoned its shell for a free-living, predatory existence, are the enormous neurophysiological and behavioral changes required to run the system.

Even though a great deal of research has been done on cephalopod behavior, to date it has been limited mostly to discrimination behavior, centered on a single sensory system such as the visual system of shape and brightness discrimination. Further, most of this work has been done on a single species, *Octopus vulgaris* (see Wells, 1978 and Boyle, 1983 for references). This pioneering work has laid the foundation for a new kind of behavioral research—call it behavioral ecology, ethology or whole-animal behavior. These comprehensive behavioral studies must be carried out in the field whenever possible, supplemented by laboratory studies for confirmation and extension of field observations. Conversely, results of laboratory studies must be compared with parallel studies in the natural habitat. Laboratory studies on captive or reared animals, however, always must recognize the potential for aberrant or adaptive behavior, thus verifying the importance of *in situ* studies for validation of laboratory observations.

At the same time, we recognize that verification of laboratory results in the field may be impossible to obtain. The pioneering work on functional behavior of bioluminescence in cephalopods, fishes, and crustaceans by R. E. Young in Hawaii is a case in point (Young and Roper, 1976, 1977; Young, et al., 1979; Young and Mencher, 1980; Young, 1981; Young, et al., 1982). Shipboard observations and experiments are the only approach possible at this time for studying these living deep-sea animals. An exciting prospect for future research lies in the potential offered by mobile, sophisticated submersibles; it has long been our desire to test our observations on counterillumination and other bioluminescent phenomena in the deep sea. I am confident this will happen.

Whole-animal behavioral studies will provide information on precisely how certain cephalopods fit in to specific marine communities; for example, what is the role and significance of octopus in intertidal and subtidal communities (Ambrose, 1982)? We believe they are the keystone predators in these habitats and perhaps in deeper habitats, as well. New, comprehensive, *in situ* behavioral studies will help answer these questions, as well as provide information on breeding, reproduction, abundance, niche partitioning, etc.

As important as these studies will be, however, they will be limited primarily to benthic, epibenthic and near shore-species and as such will concentrate on octopods, sepiids, sepiolids, and loliginids. Certainly there will be no lack of research opportunities! I point this out to indicate that a significant proportion of cephalopods, the oceanic, neritic, and deep-sea forms, will not be studied in the detailed way proposed for the other forms because they are still largely inaccessible in a technological sense. These animals will be studied at the populational and regional levels rather than at the individual and community levels. Research will be concerned with ecology, population dynamics, and resource development and utilization.

I referred to *Illex illecebrosus* earlier in relation to systematic problems and how they relate to other research and to fisheries. Acquisition of knowledge about neritic and oceanic species is absolutely dependent upon one critical factor whether the research is conducted by a systematist, ecologist, biological oceanographer or fisheries biologist. That critical factor is sampling. Regardless of the target species and the kind of data required, we cannot advance our knowledge without carefully designed and executed sampling programs. Quantitative sampling programs must account for such factors as the nature of the data desired, the size and behavior of the species involved, geographical and vertical distributions, oceanographic parameters, net avoidance, patchiness, sampling gear characteristics, mode of fishing, etc. Paramount here, of course, is the availability of modern, well-equipped research ships. These problems in relation to cephalopods have been addressed in the literature (Clarke, 1977; Roper, 1977; Okutani and Watanabe, 1983; Wormuth and Roper, 1983; Vecchione and Roper, 1985), but their importance cannot be overemphasized.

Future perspectives for research in oceanic cephalopods are very encouraging so long as objective, critical, and comprehensive quantitative sampling regimes are established. Existing technology might not be applicable to cephalopods, so new techniques and procedures may have to be developed to solve unique problems, e.g., sonic techniques for assessing harvestable populations. Then the much-needed answers will be forthcoming about breeding and spawning sites and times; spatial and temporal abundance of hatchlings, early life stages, and adults; seasonal and diel distribution; population and stock assessment; prey-predator relationships; interrelationships with other cephalopods and other groups, e.g., fishes, crustaceans; parasites and epibiots.

Laboratory Maintenance and Culture. A rapidly developing field of research on cephalopods holds great promise for the future, that of laboratory maintenance and culture of cephalopods. Boletzky and Hanlon (1983), Hanlon, et al. (1983), Hanlon and Forsythe (1985), Bergstrom and Summers (1983), Boletzky (1983) have thoroughly reviewed the subject. Loliginid and sepiolid squids and octopuses now can be cultured through several generations. Initially, intensive research on developing capture and maintenance techniques, followed by culturing techniques, was intended to provide a steady, year-round supply of squid giant axons for neurophysiological research. These objectives have been accomplished to the extent that it now seems realistic to use laboratory cultured squids for biomedical and neuroscience research. Research upon the squid third-order giant axon has led to the understanding of the mechanism of the nerve impulse, and more recently it has been used as a model of the cell membrane. These results have widespread relevance to nervous and cellular function in other phyla and the squid giant axon continues to be used in over 70 percent of all large-axon neuroscience research (R. Hanlon, pers. comm.). Other components of the squid nervous system are becoming prominent research objects, including the giant synapse as a model for studying mechanisms of synaptic transmission at the molecular level (Charlton, et al., 1982); the vertebrate-like eye for physiological and biochemical studies (Messenger, 1981); the statocysts, best developed of all invertebrates (Budelmann, 1979; Colmers, et al., 1983); the chromatophores for unique functional morphology and physiology (Hanlon, 1982; Packard, 1982). Learning and behavior studies also have begun on squid (Flores, 1983).

Similar success has been achieved with octopuses so that large-scale octopus culture now is feasible (R. Hanlon, pers. comm.; Itami, et al., 1963). Until now research on octopuses in the U.S. has been minimal because indigenous species do not occur in large, easily accessible populations. In the Mediterranean, however, abundant supplies of *Octopus vulgaris* to the laboratories at Naples, Italy and Banyuls, France have made this species the best studied of all cephalopods and possibly of almost any other marine invertebrate; its anatomy, physiology, and behavior are especially well known and *O. vulgaris* has become the experimental model for studies on a vast array of biological mechanisms (see reviews in Wells, 1978 and Mangold, 1983). Octopuses are particularly well suited to research on correlations between behavior and the nervous system because they are the only invertebrate with a closed circulatory system (Wells, 1983) and a highly vascularized brain in which the lobes are separated (Young, 1971). Octopuses make excellent experimental animals because they are easily narcotized for long periods, they withstand operative procedures, and they recover and heal readily.

Research using octopuses undoubtedly will continue to increase, particularly in learning experiments because their behavior is similar to vertebrates, they are adaptable to reward and punishment experiments, and they possess long- and short-term memory stores (R. Hanlon, pers. comm.). Hanlon also lists other research prospects for octopus that include studies of blood-brain barrier physiology, chemical transmission in the central nervous system, immune mechanisms, toxicology, eye physiology, angular acceleration, chromatophores and color patterning, nerve regeneration, endocrinology, and aging.

The proven techniques of culturing squids and octopuses open the door to many types of research, both on the biology of the cephalopods themselves, as well as using them as comparative models.

Finally, with the first indications of depletion of wild stocks of cephalopods now being reported, we may need to reevaluate the potential for mariculture of cephalopods for human consumption. Currently, mariculture is feasible only for biomedical research purposes, but it could become feasible for specialty food production within the decade where demand and price are very high and supply is low (Hanlon, In press).

Fisheries. Future development of cephalopod fisheries will increase markedly and specialized research will be necessary to insure high quality protein source at reasonable prices. In addition to the biological information required, as pointed out above, research is needed to aid in economically and safely harvesting the highest quality product. For example, an understanding of schooling and defensive behavior patterns and responses relative to fishing gear may lead to the development of new techniques to aggregate and harvest schools quickly and efficiently, just as currently is done with the squid jigging techniques. A great need exists to develop selective (species-specific) fishing gear to insure capture of target species, instead of having mixed species catches that are inefficient, damaging to the soft-bodied cephalopods, and indiscriminate in capturing abundant and depleted species alike (W. Rathjen, pers. comm.). Further, more attention must be paid to on-board handling of cephalopod catches, to insure the highest quality product. While most of the fisheries for cephalopods in the United States have concentrated on species of *Illex* and *Loligo* squids, a 3-year research program investigating the potential for an *Octopus* fishery using selective gear currently is underway on the Florida Gulf Coast (G. L. Voss, pers. comm.). Finally, an educational process is required to inform some people, primarily in North America and northwestern Europe, that cephalopods are a superior food source. Fisheries biologists and cephalopod behaviorists, systematists, and biologists will need to work together to achieve these objectives.

Research on cephalopods is at an exciting point in its development. Much has been accomplished, yet many important challenges remain. CIAC can provide guidance and analysis to point out areas of need and most productive directions in cephalopod research. The expansion of traditional research combined with the aplication of new techniques will lead to valuable increases in knowledge about cephalopods, their role in the natural world, and their potential for utilization by mankind.

So, if we need an answer to the question raised in the title of this paper, I believe it will be a resounding, "Yes!"

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PATHOLOGY OF SHIPWORMS IN BARNEGAT BAY - A MODEL SYSTEM

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ABSTRACT

Over the past nine years, four species of teredinid woodboring mollusks (shipworms) have been collected in Barnegat Bay, New Jersey, including three species of the genus *Teredo: T. navalis, T. bartschi* and *T. furcifera*, and *Bankia gouldi*. The total abundance of shipworms, greatest during the first year of the study, has declined in recent years, with two of the *Teredo* species, *T. furcifera* and *T. bartschi* having disappeared altogether from collections in the bay. It was found that protozoan parasites in the shipworms may have been responsible in part for the population fluctuations.

The *Teredo* species are commonly infected with an ascetosporan, *Haplosporidium* sp., while individuals of *B. gouldi* are less often found containing encysted ciliates, *Boveria teredinidi*. It was demonstrated that *Teredo* abundance tended to decrease with increasing infection levels of *Haplosporidium* sp., but that the various *Teredo* species may differ in their resistance to the parasite. On the other hand, no correlation between increased encystment of *B. teredinidi* and declines in abundance of *B. gouldi* could be shown. However, *B. teredinidi* is generally an ectocommensal on both *B. gouldi* and *Teredo*, and is usually unable to invade shipworm tissues. No such invasion has been found in *Teredo*, yet it happens in about 10 percent of all *B. gouldi* found in Barnegat Bay.

The variability in resistance to *Haplosporidium* sp. among the *Teredo* species, and the lack of encystment, a common molluscan defensive reaction against non-self materials, in *Teredo* provide opportunities for sophisticated comparative studies of molluscan defense mechanisms.

A heavy outbreak of woodboring mollusks (Family Teredinidae), commonly called shipworms, in the vicinity of a power generating station in Ocean County, New Jersey, in the early 1970's raised concerns about the possible effects of the plant's thermal discharge on populations of woodborers in Oyster Creek, into which the power plant discharges, and in the Barnegat Bay system as a whole. A long-term study, still ongoing, was begun in 1975 to determine whether the power plant was indeed impacting on the abundance, distribution and/or reproductive patterns of any of the several species of shipworms found in Barnegat Bay.

Population parameters are determined from collections of the woodborers in untreated wood panels located at various sites around Barnegat Bay. Originally, panel arrays were located at 17 sites. During the ensuing years, some locations were changed slightly,and a few collection sites were added so that borers are now being collected at 20 stations (Fig. 1).

The sampling technique consists of mounting seven 1.9 x 8.9 x 25.4 cm untreated soft pine panels on an iron rack (Fig. 2) and submerging the rack vertically below the low tide level to a point several centimeters above the sediment-water interface at each site. The panel racks placed at each site contain one short-term panel (labeled "C") and six long-term panels (numbered 1 through 6). Two larger special panels (unlabeled) are also mounted on each rack, along with two panels which have been creosoted for another aspect of the overall study.

Each month, the short-term panel and one of the long-term panels are removed and replaced. Long-term panels are removed sequentially such that each has been exposed for six months at the time it is removed. The two special panels are removed and replaced annually in May and June.

The removed panels are analyzed microscopically and macroscopically in the laboratory for the presence of shipworms. Short-term panels indicate the number of each species of borer that settles each month, and provide information on the range of months over which settlement takes place. Long-term panels allow determination of growth over the six-month period as well as annual

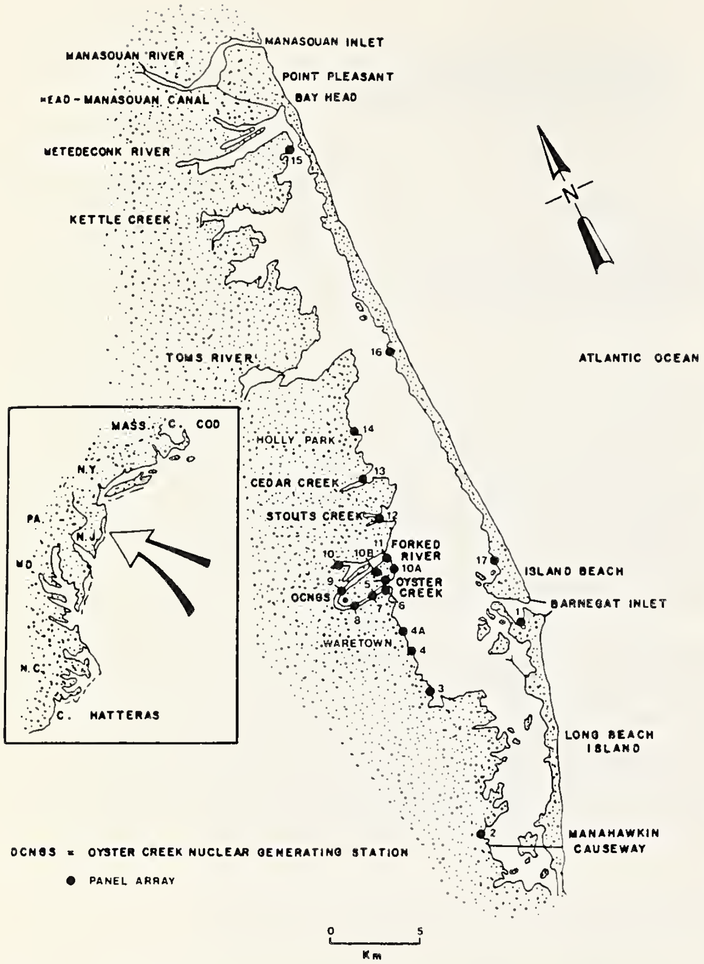

Fig. 1. Map of Barnegat Bay showing geographic locations of exposure panels.

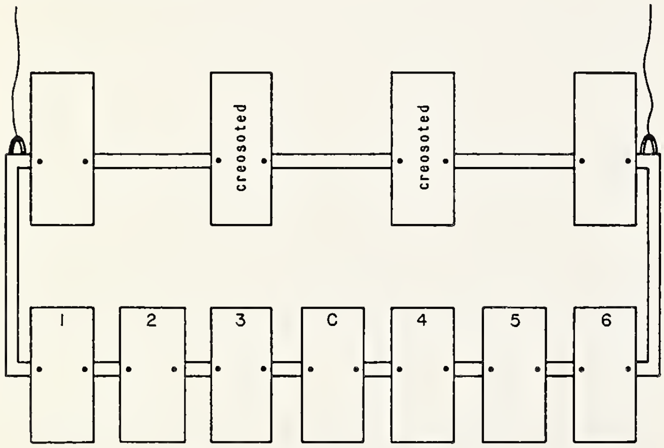

Fig. 2. Exposure Panel Array.

and seasonal variations due to fluctuations in water temperature, salinity and/or other environmental parameters. The two larger panels provide specimens for gonad analyses during the months when no shipworms would be expected in the regular panels.

For gonad development studies, shipworms are removed from the panels, fixed, and embedded in a paraffin compound for thin sectioning.

The first series of panels, installed in June, 1975 and examined monthly throughout the summer and fall of 1975, resulted in the identification of at least four species of shipworms in the bay. Three species were of the genus *Teredo: T. navalis* Linné, *T. bartschi* Clapp, and *T. furcifera* von Martens. The fourth and most abundant and widely distributed of the four species at the end of the first full breeding season was *Bankia gouldi* Bartsch. This species has occurred at some time during the study at each of the panel locations, and at the end of 1975 and the beginning of 1976, accounted for almost half of all shipworms collected throughout Barnegat Bay.

Table 1. Numbers of teredinid borers in long-term exposure panels exposed in Barnegat Bay, New Jersey from July, 1975 through June, 1984.

Bioyear	*T. navalis*	*T. bartschi*	*T. furcifera*	*B. gouldi*	Total
75-76	1,110	5,507	381	11,400	18,398
76-77	2,342	15	4	505	2,866
77-78	2,255	771		460	3,486
78-79	2,145	2,785		246	5,176
79-80	2,872	2,918		1,196	6,986
80-81	1,404	103		292	1,799
81-82	1,481	1,075		101	2,657
82-83	1,232			52	1,284
83-84	953			606	1,559

Table 1 shows the numbers of teredinid borers of each species collected in long-term panels each "bioyear" from July, 1975 through June, 1984. A bioyear is the period from July of one year through June of the next, and corresponds to the breeding season of teredinids in Barnegat Bay (Richards *et al.*, 1979).

The total abundance of shipworms was greatest during the first bioyear of the study, and fell off sharply during the second year. At no time during the remainder of the study has the total number of shipworms been close to what it was during the first bioyear, despite the fact that three more collecting sites are now being used than were operative during that first year.

Two species were primarily responsible for the large number of shipworms during the 1975-76 season. *B. gouldi* alone accounted for 11,400 shipworms, more than three times the total number of *B. gouldi* collected throughout the next eight seasons. *T. bartschi* during that same period was represented by over 5,500 individuals, almost twice as many as any species of *Teredo* in any other bioyear. Since that first year, *B. gouldi* has shown a significant decline (Hillman *et al.*, 1983) in abundance in spite of occasional peaks. *T. bartschi* has apparently disappeared from the bay altogether. *T. furcifera* also has not been collected since early 1977, despite being very abundant during the summer of 1974 (Hoagland and Turner, 1980).

Teredo bartschi and *T. furcifera* are both subtropical species which may have been introduced into Barnegat Bay in wooden boats coming up from the south (Hoagland and Turner, 1980). *T. bartschi* was sustained in the warm discharge of the power plant (Hoagland and Turner, 1980). *T. furcifera*, on the other hand, was also collected at sites not affected by the power plant's discharge (Richards *et al.*, 1980).

Richards *et al.* (1976, 1979) ascribed the marked decrease in the abundance of *B. gouldi* which occurred after the 1975-76 season to the severe winter of 1975-76 (during which many of the overwintering individuals were killed), to natural teredinid cycles, and, in Oyster Creek, to the additional effect of the removal of all untreated wood by the Jersey Central Power and Light Company during the spring of 1975 and winter of 1975-76. These same factors could also have had a mitigating effect on *T. bartschi* and *T. furcifera* as well. With the power plant not operating through most of 1983 and 1984, winter water temperatures in the discharge area may have been too low for *T. bartschi* to survive, and it, too, has disappeared from the bay along with *T. furcifera*.

It now appears that there also has been a significant decline in the *T. navalis* population since the 1979-80 season, that can not necessarily be explained by temperature and/or salinity alone (Hillman *et al.*, 1984). One factor that may have contributed significantly to teredinid mortality in the bay, and thereby to the decline, is the effect of certain protozoan parasites of the shipworms. It was mentioned above that histological analyses of gonad developmental patterns are part of the overall investigation of shipworm populations in Barnegat Bay. During the course of these studies, two protozoan species were discovered to be parasitic in the shipworms. One was an ascetosporan, *Haplosporidium* sp., which was found in all three species of *Teredo* in the bay (Hillman, 1978). The other was a ciliate, *Boveria teredinidi* Nelson, not normally known to be anything but an ectocommensal in Barnegat Bay shipworms (Nelson, 1923; Pickard, 1927), but which was frequently found encysted in the tissues of *B. gouldi* in Barnegat Bay (Hillman, 1979).

At least the ascetosporan may have had a direct affect on populations of *Teredo*. Hillman *et al.*, (1982), for example, showed that the abundance of *T. navalis* at selected sites in Barnegat Bay was reduced in bioyears following levels of infection of 40 percent or more by *Haplosporidium* sp. In addition, this parasite, which was also found in *T. bartschi* and *T. furcifera*, may have acted synergistically with the other environmental parameters mentioned above to cause the complete disappearance of those species from Barnegat Bay.

The effects of the ciliate are not as clear cut. Other environmental factors may be operating to limit populations of *B. gouldi*, and these factors may be indirectly manifested in the encystment of individual *Boveria teredinidi* in the tissues of *B. gouldi*.

The epizootiological effects of the two protozoans on the Barnegat Bay teredinids will be looked at in more detail below, and some interesting opportunities to investigate more basic molluscan pathology afforded by these host-parasite relationships will be discussed.

HAPLOSPORIDIUM SP. IN *TEREDO*

From August, 1975, through November, 1984, a total of 2,869 individuals of the genus *Teredo* from Barnegat Bay were examined histologically for the presence of life-cycle stages of the ascetosporan *Haplosporidium* sp. This includes 2,248 *T. navalis*, 534 *T. bartschi*, 24 *T. furcifera*, and 62 immature teredinids too small to be identified to species. *Teredo* species were collected from each of the 20 stations at least once during the study, and infec-

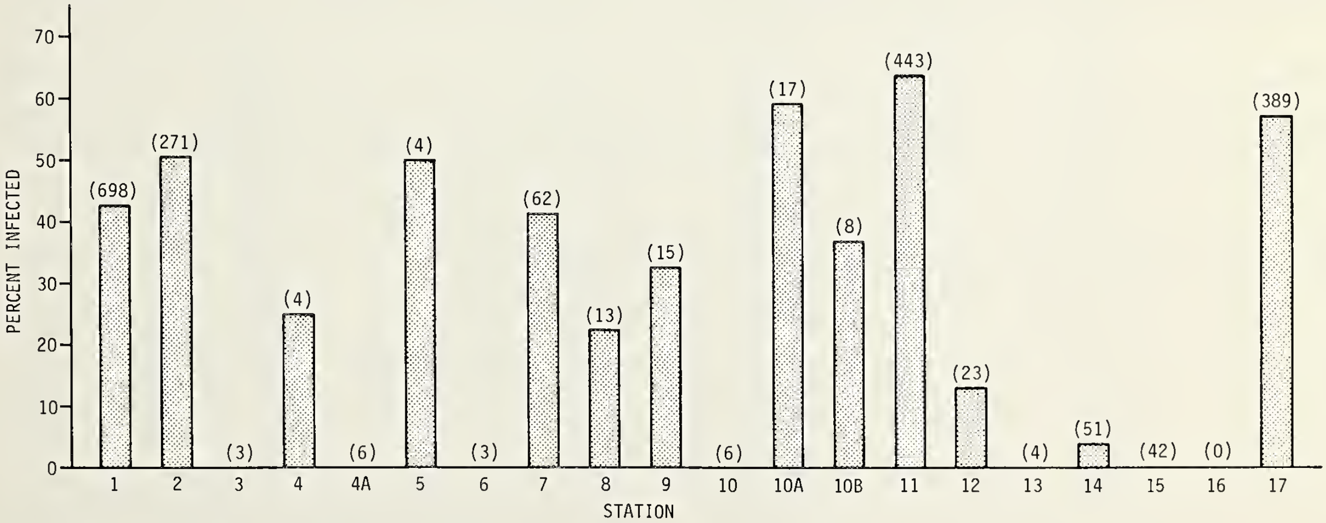

Fig. 3. Percentage of *Teredo navalis* infected with *Haplosporidium* sp. at each station in Barnegat Bay from July, 1975 through November, 1984. Numbers in parentheses indicate number of individuals examined.

tions occurred at all but six stations: Stations 3, 4A, 10, 13, 15 and 16. Infection levels were relatively high at most stations, but only at Stations 1, 2, 11 and 17 were substantial numbers of shipworms involved (Fig. 3).

Most *T. navalis* occurred at Station 1, which was in Barnegat Inlet (Fig. 1), and which may have drawn as many settling larvae from outside the bay as inside. The infection rate of about 43 percent (Fig. 3) at that station was the lowest rate found at any of the four heavily populated stations, and the abundance of shipworms in long-term panels there fluctuated less. However, there was a considerable decline in abundance at Station 1 following the 1979-80 bioyear, and infection rates were generally higher since that period (Table 2).

Table 2. Abundance of *Teredo navalis* in long-term panels at selected stations in Barnegat Bay and rate of infection by *Haplosporidium* sp.

Station	Bioyear	Abundance[1]	Infection[2] Rate
1	75-76	480	0/4
	76-77	2191	13/44
	77-78	2225	8/70
	78-79	2105	30/78
	79-80	2119	35/71
	80-81	1261	77/116
	81-82	1418	36/90
	82-83	810	50/77
	83-84	849	1/22
2	75-76	25	3/6
	76-77	4	0/0
	77-78	4	0/3
	78-79	21	2/22
	79-80	109	88/147
	80-81	36	27/36
	81-82	0	-
	82-83	0	-
	83-84	2	0/20
11	75-76	259	1/2
	76-77	84	12/31
	77-78	10	4/4
	78-79	13	0/6
	79-80	529	5/57
	80-81	73	46/84
	81-82	30	27/34
	82-83	309	168/189
	83-84	63	0/7
17	75-76	313	14/19
	76-77	53	10/16
	77-78	5	7/8
	78-79	5	7/16
	79-80	22	34/46
	80-81	14	33/158
	81-82	10	6/14
	82-83	68	96/148
	83-84	71	67/179

[1] From long-term (6-month) panels only
[2] From 6-month and 12-month panels

At Station 2, one of the stations least affected by incoming currents through Barnegat Inlet, *T. navalis* abundances fluctuated widely throughout the study. During the first bioyear, only 25 *T. navalis* were collected from long-term panels at that site. Half of the specimens examined, however, were infected with *Haplosporidium*. Over the next three bioyears, a total of 29 *T. navalis* were collected at Station 2. No *Haplosporidium* infections were found until the 1978-79 bioyear, when the infection rate was less than 10 percent. By the following bioyear (1979-80), shipworms reached their peak abundance at Station 2.

Station 11 is another site where the abundance of *T. navalis* fluctuated widely over the years, as has the infection rate of *Haplosporidium*. For the two years after the first bioyear, the abundance of *T. navalis* dropped sharply, while the infection rate rose. During the third bioyear, still only a few *T. navalis* were collected in long-term panels at Station 11, but none of the individuals examined showed indications of *Haplosporidium* infections. The following year (1979-80) saw more *T. navalis* in the long-term panels than the total found during the four previous bioyears, and the infection rate was still low. The abundance of *T. navalis* at Station 11 dropped during the 1980-81 bioyear and remained low through the 1981-82 bioyear, while *Haplosporidium* infections increased. Although *T. navalis* increased in abundance during the 1982-83 bioyear, the infection rate was close to 90 percent, and by the end of the next bioyear, the number of *T. navalis* collected in long-term panels at Station 11 was again down sharply.

At Station 17, 313 *T. navalis* were collected in long-term panels during the first bioyear, and only a total of 248 during the next eight bioyears. The infection rate ranged from a low of about 21 percent up to 87 percent, and was

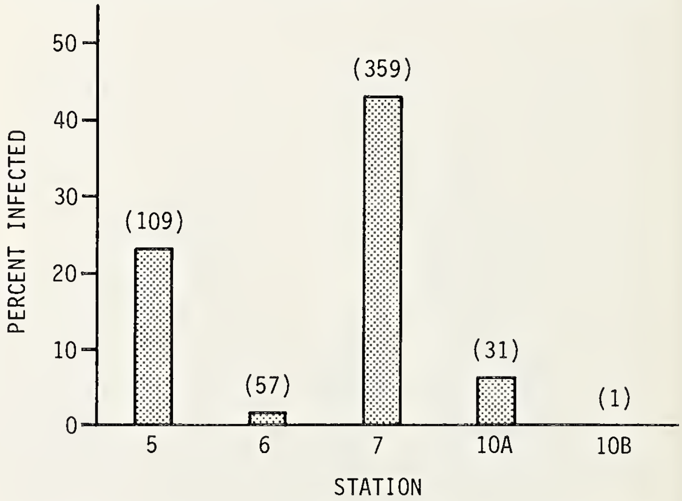

Fig. 4. Percentage of *Teredo bartschi* infected with *Haplosporidium* sp. in Barnegat Bay from July, 1975 through March, 1982. Numbers in parentheses indicate number of individuals examined.

over 40 percent for seven of the nine bioyears of the study.

Overall infection rates in *T. bartschi* (Fig. 4) were, on the average, considerably lower than in *T. navalis*. The highest infection rate of about 43 percent occurred at Station 7 where most *T. bartschi* were collected. Infection rates in both *T. navalis* and *T. bartschi* were about the same at Station 7.

Table 3 shows the abundance of *T. bartschi* in long-term panels during each bioyear at each station at which it occurred, and the infection rate. *T. bartschi* was very abundant in collections from Stations 5 and 6 during the first bioyear, and then declined, almost disappearing, until late 1977 when it increased, primarily at Station 7. *Haplosporidium* infections were not observed at Stations 5 and 6 until late in 1981, and were light at Station 7 until the 1980-81 bioyear. The possibility of resistance to *Haplosporidium* by *T. bartschi* was suggested by Hillman *et al.* (1982), but infections increased markedly by early 1982, and by the end of 1982, *T. bartschi* had disappeared from the bay. While *T. bartschi* may not be completely resistant, it is possible that it is more so than *T. navalis*. Its disappearance from the bay may be more related to the power plant not operating than to the high infection rates seen during the 1981-82 bioyear.

There is an apparent seasonal nature to the infections, at least in *T. navalis*. Infection rates have been lowest in August and tended to build up through the fall (Fig. 5). Peak infection rates occurred in November, December and January.

It was stated earlier that at selected stations, the abundance of *T. navalis* in long-term panels could be expected to decrease in the bioyear following an infection level of 40 percent or more (Hillman *et al.*, 1982). When the total abundance of *T. navalis* for each bioyear from July, 1975 through June, 1984 is plotted with the number of shipworms infected with *Haplosporidium*, estimated on the basis of the overall infection rate for each bioyear (Fig. 6), it can be seen that as the rate of infection decreased, the overall abundance throughout the bay, at least on long-term panels, declined.

BOVERIA TEREDINIDI IN *BANKIA GOULDI*

From July, 1975 through November, 1984, a total of 2,615 *Bankia gouldi* from 20 stations in Barnegat Bay were

Table 3. Abundance in long-term panels and infection rate of *Teredo bartschi* in Barnegat Bay from July, 1975 through June, 1983.

	STATION									
BIOYEAR	5		6		7		10A		10B	
	NO.	% INF.	NO.	% INF.	NO.	% INF.	NO.	% INF.	NO.	% INF.
75-76	3435	-	1676	0	0	-	*		*	
76-77	0	-	0	-	15	40	*		*	
77-78	1	-	2	-	768	39	0	-	0	-
78-79	532	0	87	0	2166	0	0	-	0	-
79-80	1164	0	466	0	1107	0	179	0	1	0
80-81	0	0	10	0	93	52	0	-	0	-
81-82	108	76	2	50	965	82	0	-	0	-
82-83	0	-	0	-	0	-	0	-	0	-

* No panels

Table 4. Abundance of *Bankia gouldi* in long-term panels at selected stations in Barnegat Bay, and rate of encystment of *Boveria teredinidi*.

	STATION											
	7		10A		11		12		13		14	
Bioyear	Abundance[1]	Rate[2]	Abundance[1]	Rate[2]	Abundance[1]	Rate[2]	Abundance[1]	Rate[2]	Abundance[1]	Rate[2]	Abundance[1]	Rate[2]
75-76	14	0/8	*		1859	11/22	205	3/32	1702	9/22	2343	6/11
76-77	21	0/1	*		181	0/22	36	3/19	129	23/32	68	6/22
77-78	30	0/28	*		255	3/47	30	5/30	58	2/28	36	1/30
78-79	10	0/3	16	3/15	73	0/56	3	0/3	41	0/22	70	0/31
79-80	5	0/8	135	2/40	425	30/176	109	2/164	177	5/61	256	7/90
80-81	6	1/27	57	10/40	108	10/143	65	9/214	7	0/7	4	0/4
81-82	4	2/6	9	0/11	15	2/33	10	0/39	10	0/12	37	2/41
82-83	0	0/3	2	0/3	4	1/23	1	0/7	13	0/15	8	0/12
83-84	18	1/46	8	0/8	199	13/196	9	1/31	69	8/57	248	25/106

[1] From long-term (6-month) panels only

[2] From 6-month and 12-month panels

* Station 10A not established until the 1978-79 bioyear

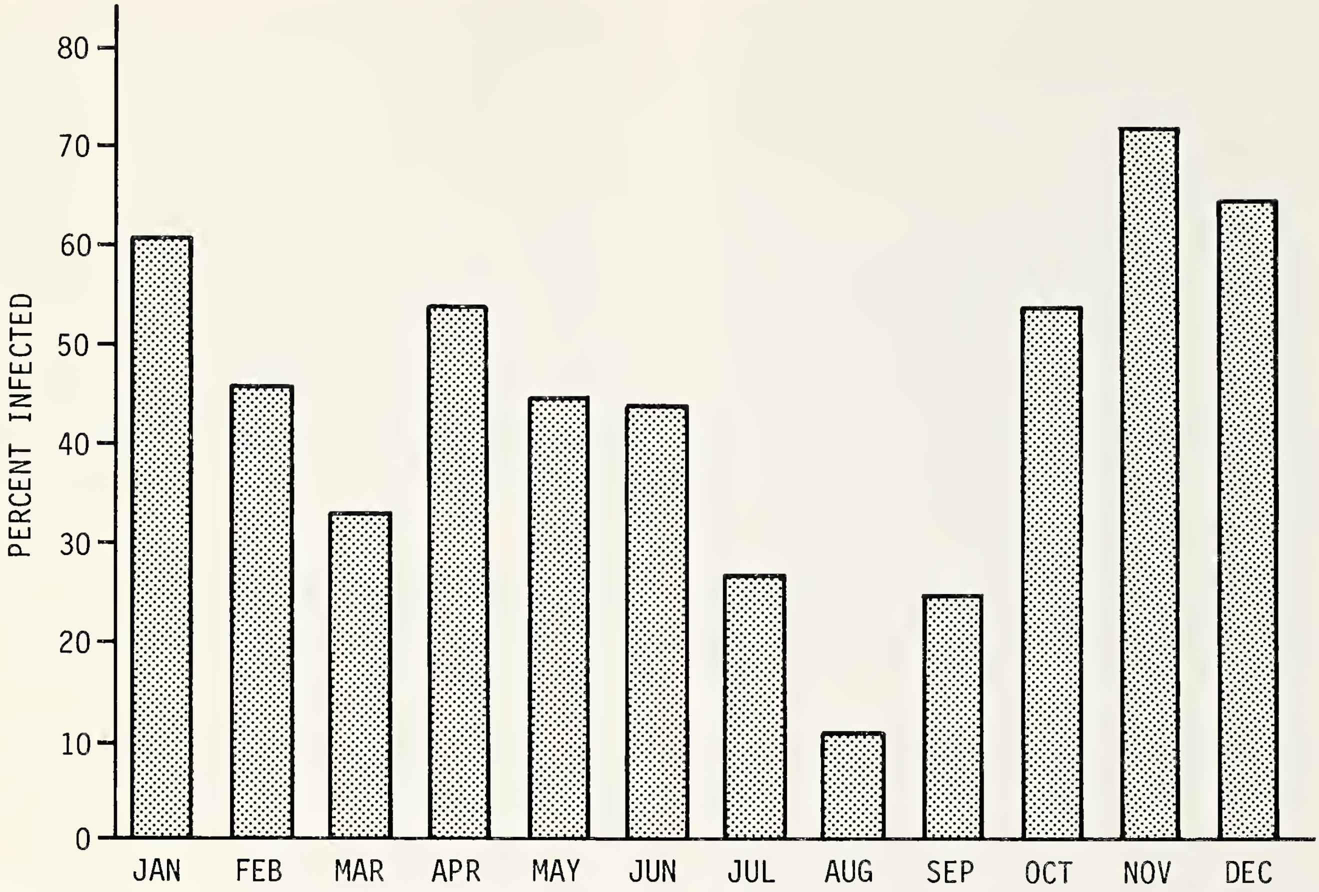

Fig. 5. Total monthly rates of infection of *Teredo navalis* by *Haplosporidium* sp. in Barnegat Bay, New Jersey from July, 1975 through November, 1984.

examined histologically. About 11 percent of them contained one or more encysted *Boveria teredinidi*. Only at Stations 1 and 17, on the eastern side of the bay where salinities tend to be higher (e.g., Hillman *et al.*, 1984), did no encystment occur (Fig. 7). The highest encystment percentage, about 33 percent, occurred at Station 6, but only a relatively few shipworms were involved. Encystment rates were highest, from 21 to 26 percent, during the first two bioyears of the study (Fig. 8). As the abundance of *B. gouldi* around the bay declined, so did the encystment rate, with the lowest rate being recorded during the 1982-83 bioyear. Only 52 individuals were collected in long-term panels during that bioyear, and only about one percent of individuals examined contained encysted *B. teredinidi*.

Although *B. gouldi* occurred at least once at all 20 stations during the nine bioyears of study, most were collected from only six stations: Stations 7, 10A, 11, 12, 13, and 14. Table 4 shows the number of *B. gouldi* collected in long-term panels and the rate of *Boveria* encystment at those stations during each bioyear of the study. Annual infection rates generally reflected those shown for the entire study, but occasionally they were unusually high. At Station 13 during the 1976-77 bioyear, for example, about 72 percent of *B. gouldi* examined contained encysted *Boveria*. At no station has encystment occurred during every bioyear, but throughout six of nine complete bioyears, encystment has occurred at Stations 11, 12, and 14.

As with *Haplosporidium* sp. in *Teredo*, there appeared to be a seasonal cycle of *Boveria* encystment, with most cysts observed during the fall and early winter (Fig. 9).

The pathological effects of encapsulation of *Boveria* in *B. gouldi* were described previously (Hillman, 1979). Cysts were found in all tissues, but most occurred in the gills and digestive gland. The process of encapsulation was the hemocytic type described by Cheng and Rifkin (1970). Encystment was often accompanied by considerable hemocytic infiltration into the tissues in which the cysts occurred. Frequently, enlarged and extremely eosinophilic hyalinocytes were found in the immediate area of the cyst. Occasionally, columnar epithelium in any of the tissues in which the cysts occurred was reduced to low cuboidal, and there was often extensive hemocytosis and metaplasia of epithelial layers without any evidence of encapsulation having taken place.

As traumatic as the cysts appeared, there did not seem to be any direct relationship between levels of en-

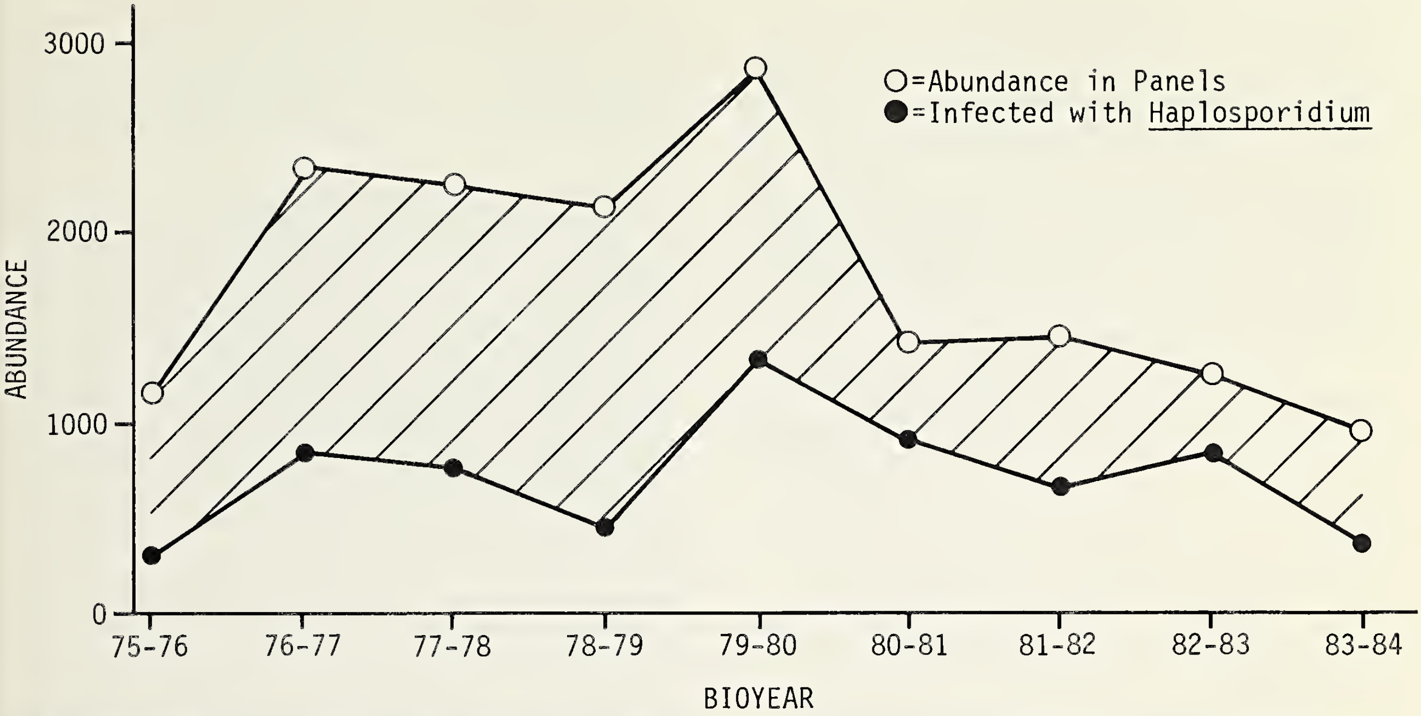

Fig. 6. Relationship of abundance of *Teredo navalis* in long-term exposure panels to numbers infected with *Haplosporidium* sp. Based on infection rates in Barnegat Bay, from July, 1975 through June, 1984.

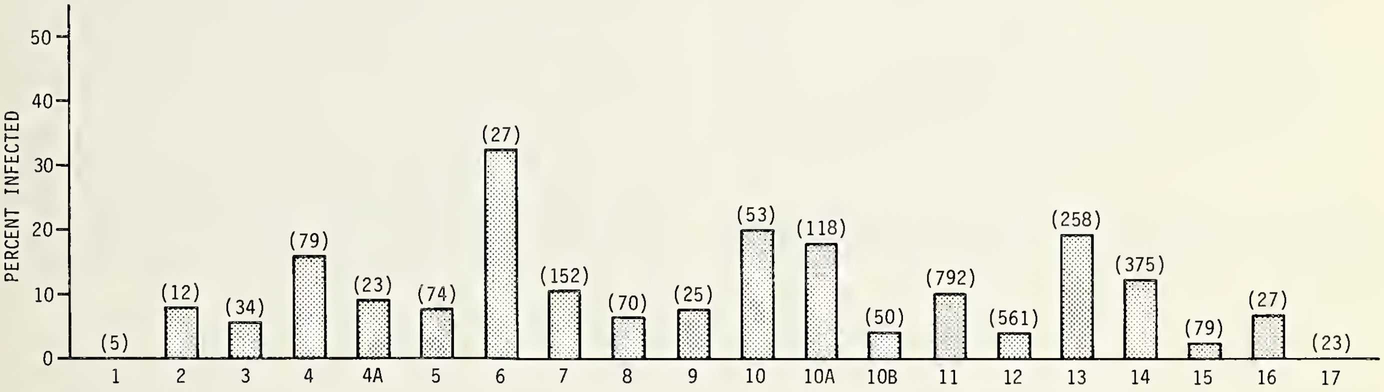

Fig. 7. Percentage of *Bankia gouldi* with encysted *Boveria teredinidi* at each station in Barnegat Bay from July, 1975 through November, 1984. Numbers in parentheses indicate number of individuals examined.

cystment and fluctuations in abundance of *B. gouldi* in long-term panels.

SHIPWORMS AS MODEL PATHOLOGY STUDY SYSTEMS

Both the *Haplosporidium* sp. infections in the various species of *Teredo* and the encystment of *Boveria teredinidi* in *Bankia gouldi* raise some interesting questions regarding the basic nature of molluscan defense mechanisms. In mollusks, as well as other animals, defense reactions at the tissue level depend on the ability of the host to distinguish its own cells from foreign cells or material. In the case of *Haplosporidium* infection, resistance, once the parasite is recognized as foreign, may be related to increases in hemolymph lysozyme concentrations, as speculated upon by Feng and Canzonier (1970) for *Haplosporidium nelsoni* (Haskin, Stauber and Mackin) infections in the oyster.

At one point, *Teredo bartschi* populations in Barnegat Bay may have developed a resistance to *Haplosporidium* infections. *T. navalis* at the same or nearby stations was found with high levels of infection while *T. bartschi* at two stations went completely uninfected for five to six years, and had relatively light infections at another station. Then suddenly during the 1981-82 bioyear, extemely high infection rates occurred in *T. bartschi*, and by the next year *T. bartschi* was gone from Barnegat Bay. It is quite likely that low ambient water temperatures were ultimately responsible for the disappearance of *T. bartschi* but did those temperatures have an effect of lessening the resistance of *T. bartschi* to the *Haplosporidium* infections? Is *T. bartschi* more resistant to begin with? Overall infection rates were never as high in *T. bartschi* as in *T. navalis*. Having two species of *Teredo*, and possibly three, in Barnegat Bay affords the opportunity for some sophisticated comparisons of *Haplosporidium* infection and resistance.

It has been pointed out (Hillman 1978, 1979) that *Haplosporidium* sp. is morphologically similar, if not identical, to *Haplosporidium nelsoni*, and may indeed be *H. nelsoni*, with the various species of *Teredo* acting as reservoir hosts. If this is so, then the ability of *Haplosporidium* to infect at least three species of *Teredo* and a species of *Crassostrea*, but not *Bankia gouldi*, another teredinid, is very interesting. Again, the opportunity for comparative studies of resistance is conveniently provided.

The response of *Bankia gouldi* to invasion by the ciliate *Boveria teredinidi* also raises some interesting questions with regard to resistance by mollusks. For example, just how resistant is *B. gouldi* to penetration by the ciliate? Reports of encystment by shipworms of any invading species are very rare. Levinson's (1941) report of encystment of *Boveria zenkevitchi* Levinson in *T. navalis* in Sebastopol Bay is the only other account of encystment by shipworms. In Barnegat Bay, however, *B. teredinidi* was able to penetrate the tissues of *B. gouldi* at an average rate of almost 10 percent of the time over the past nine years, and at some locations at levels of 20 to 30 percent. Has resistance to invasion broken down in about 10 percent of the *B. gouldi* examined so that ciliates can penetrate the tissues, or is resistance weakened in a larger percentage of shipworms, with only 10 percent of the individuals examined being able to muster their defense mechanisms to encapsulate the invader? Since no instance of encystment has been observed in nearly 3,000 *T. navalis* examined over the nine-year study period, could *B. gouldi* be less resistant to invasion than *T. navalis*? Or could both species be susceptible to invasion, with only *B. gouldi* being able to react defensively to encyst the invader, whereas *T. navalis* is killed? And lastly, is this encystment phenomenon unique to Barnegat Bay?

Morphological, biochemical and behavioral comparisons of the varied defense reactions among the closely related shipworm species in Barnegat Bay might provide the key to answers of some of the most basic questions currently before invertebrate pathologists.

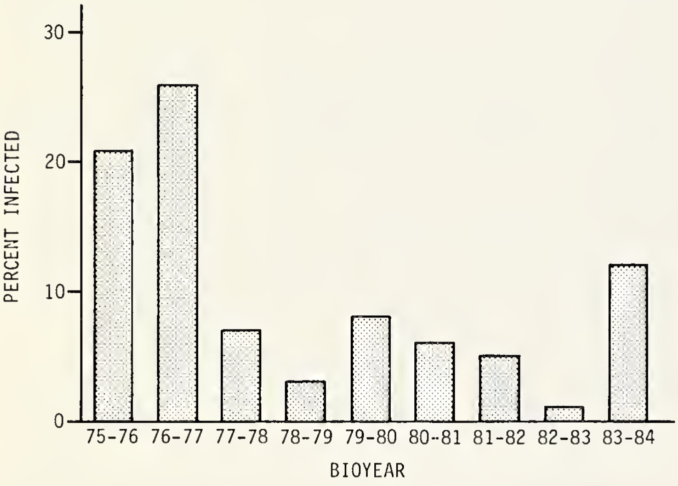

Fig. 8. Percent of *Bankia gouldi* in Barnegat Bay containing encysted *Boveria teredinidi* each bioyear from July, 1975 through June, 1984.

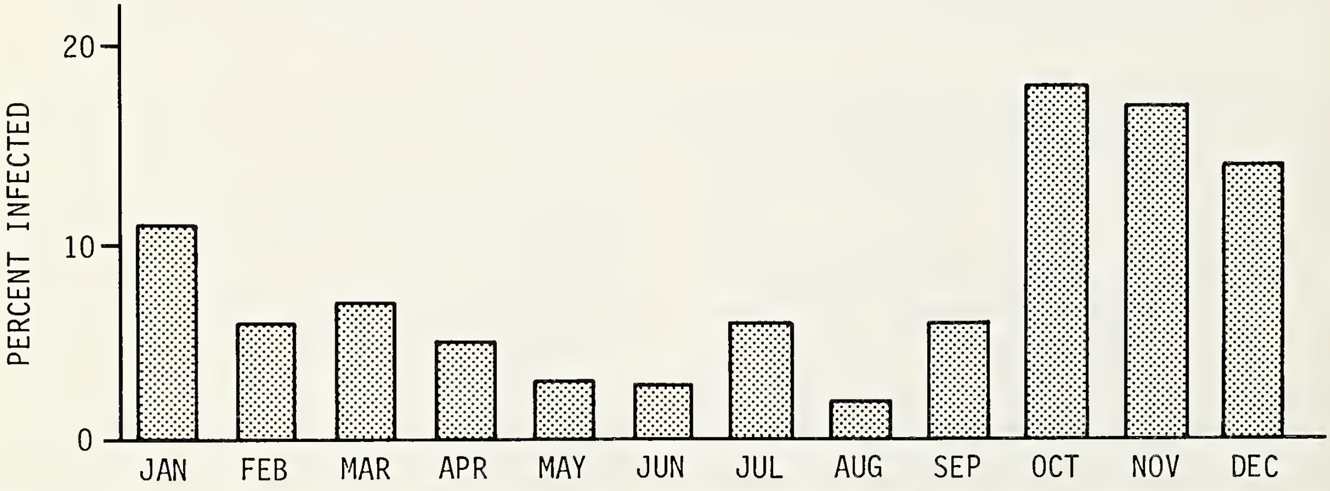

Fig. 9. Total monthly rates of infection of *Bankia gouldi* by *Boveria teredinidi* in Barnegat Bay, New Jersey from July, 1975 through November, 1984.

ACKNOWLEDGMENTS

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OYSTER DRILLS AND THE FRONTIERS OF CHEMICAL ECOLOGY: UNSETTLING IDEAS

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ABSTRACT

Chemicals associated with adult barnacle (*Balanus amphitrite*, Darwin) attract predatory snails (*Urosalpinx cinerea*, Say). Biochemical fractionation of barnacle odor resulted in a specific fraction with potent snail attractant activity. Tests with barnacle larvae indicated that the purified fraction also modified barnacle behavior and induced metamorphosis. The chemicals are attractive to predators when in solution, however, they were found to be attractive to barnacle larvae only when adsorbed to surfaces. Chemicals associated with natural stands of oysters also exhibit potent snail attractant properties. Snail attractants isolated from oysters modify behavior of larval barnacles, but *do not* induce metamorphosis. The responses of snails and larval barnacles to the biochemical fractions occurred at concentrations similar to those detected in field situations. Water from the field tested in barnacle behavior assays showed that larvae respond positively to water from areas with high barnacle density. Predatory snails have apparently tapped an originally intraspecific communication system that functions to cue larval settlement. I suggest that this type of complex and multifunctional chemical communication system may be common in marine environments.

In aquatic environments chemicals indicate presence of food, hosts, prey, potential commensal organisms, sexual partners, settlement substrates or localities, and other resources (Crisp and Meadows, 1962; Dimock and Davenport, 1971; Gurin and Carr, 1974; Miller and Tseng, 1974; Bardach, 1975; Morse *et al.*, 1979; Atema, 1980; Derby and Atema, 1980; Rittschof, 1980; Rittschof *et al.*, 1983). As knowledge of aquatic chemical sensing increases, more complex and higher order interactions involving chemical cues are becoming apparent. There is a growing literature documenting chemicals as larval settlement inhibitors, camouflaging agents, chemosensory inhibitors and facilitators, and as offensive and defensive weapons (Williams *et al.*, 1983; Standing *et al.*, 1984; Zimmer-Faust *et al.*, 1984; Rittschof *et al.*, 1985a). Although the work by Morse and his group on the biochemical control of abalone settlement is far and away the most complete and elegant work in the field, the marine ecosystem that includes barnacles, bivalves and predatory snails is the most extensively studied with respect to chemical integration. The complexity of this system (chemical attractants, facilitators, suppressants, and settlement factors) is the basis for the emerging concept of an integrated chemical habitat web. Central to that concept are specific peptide cues associated with odors (Rittschof *et al.*, 1983). Arguments as to the particular advantages of discrete substances like peptides with high information content can be found in Rittschof (1980), Rittschof *et al.*, (1984a, 1985b).

Although the origin of the peptides is still under investigation, the hypothesis guiding that work is that the active peptides were originally either excretory products or the result of hydrolysis of structural proteins. Because of the specific information content of these peptides, there is a basis for the evolution of secondary cueing functions such as prey location by predatory snails (Rittschof *et al.*, 1983). However, just as the chemicals that comprise the odor of the skunk provides several cueing functions, it is likely that specific peptide cues could provide the same information to several different kinds of animals. Thus, the same cue could elicit a variety of behaviors. Central to this concept is the biochemistry and sensory physiology common to complex organisms. By similar argument, it is likely that similar types of odor molecules from different types of organisms could perform similar functions based in part upon the life history of the organisms in question and in part upon the properties of the information containing molecules.

Some muricid gastropods, notably *Urosalpinx cinerea* (Say) use highly specific chemical cues to locate prey. They do this using a combination of flow and prey odors in solution (Carriker, 1955; Blake, 1962; Wood,

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1968; Pratt, 1974; Rittschof *et al.*, 1983; Brown and Rittschof, 1984). Chemical substances involved in prey locations have been isolated and partially characterized from seawater bathing living barnacles and oysters. In the case of barnacle odor, these substances are peptides of 3000-7000 daltons (Rittschof *et al.*, 1984a). Substances from oyster odor are similar in chemical properties to barnacle peptides (Rittschof and Shephard, unpublished data).

Gregarious settlement in barnacles is essential for reproduction in some forms (Crisp, 1965). Gregariousness is attributed in part to recognition of surface chemistry by cyprids, the larval settling stage (Knight-Jones and Stevenson, 1950; Knight-Jones, 1953; Crisp and Meadows, 1962, 1963; Crisp, 1965, 1974; Meadows and Campbell, 1972). Acidic glycoproteins (5000 to 18000 dalton subunits) that promote gregarious settlement can be extracted from homogenates of adult barnacles (Larman *et al.*, 1982). Because barnacle larvae are known to respond to protein settlement factors (Crisp and Meadows, 1963; Larman and Gabbott, 1975; Larman *et al.*, 1982; Rittschof *et al.*, 1984b) and because larval barnacles don't encounter homogenates of barnacles in the field, we decided to test the hypothesis that larval barnacles use components of adult odors as cues to settlement.

Here we report that:

1) Specific prey chemicals that function to evoke creeping behavior in predatory snails also cue reversible attachment of settling stage larval barnacles.

2) Snail creeping is evoked by the chemical components in solution, but larval barnacles behavior is modified only after adsorption of the chemicals onto surfaces. Active concentrations are within the range that can be expected *in situ*.

3) Similar chemical components from different prey (barnacles and oysters) both cue larval behavior changes, but only the conspecific cue significantly increases the percentage of larval transformation, that is facilitates metamorphosis.

I do not present this work as a completed study, but rather as an illustration of the directions that this particular aspect of chemical ecology can be expected to take in the future.

METHODS

FRACTIONED AND PARTIALLY PURIFIED OYSTER AND BARNACLE ODORS

Partially purified chemicals from water surrounding living intact oysters (*Crassostrea virginica*) and barnacles (*Semibalanus balanoides*) were a generous gift from Professors R.G. Shepherd and M.R. Carriker and the other participants of the University of Delaware Oyster Drill Project. These peptide solutions had been taken through two adsorption chromatography steps (Rittschof and Shepherd, unpublished). The first chromatography step eliminates 99%+ of contaminating compounds (Rittschof *et al.*, 1984a). The second chromatography step increases the specific activity approximately 5 fold. Dilution of the partially purified material 5,000 to 10,000 fold with seawater filtered to remove particles greater than 1 μm evoked high percentages of snail (*Urosalpinx cinerea*) creeping responses (Rittschof *et al.*, 1984a) at levels comparable to responses of snails to unfractionated chemicals. Snails responded at concentrations that were also comparable to levels of chemicals detected in field bioassays (Rittschof *et al.*, 1984b).

Balanus amphitrite amphitrite Darwin nauplii were cultured to cyprids in the laboratory for use in behavior and settlement bioassays (Rittschof *et al.*, 1984c). These were scored utilizing the sequence of events characterized by Crisp (1955, 1965, 1974) and others (Knight-Jones and Stevenson, 1950; Knight-Jones, 1953; Crisp and Meadows, 1962, 1963; Meadows and Campbell, 1972).

LARVAL BEHAVIOR ASSAY

The behavior assay was a 2.5 min assay that used flow to promote cyprid investigation of the inner surface of a 3.5 mm i.d. by 12 cm Pyrex glass tube (Crisp, 1955). Prior to assay, a tube was rinsed in seawater (control) or with a test solution containing adult barnacle or oyster chemical fractions. The settlement factor (Larman *et al.*, 1982) treatment, a mixture of proteins from homogenates of barnacles, was at 10 μg protein ml^{-1} (Rittschof *et al.*, 1984c). Partially purified barnacle odors were tested at 2.5 μg ml^{-1} and at levels that evoke creeping responses in snails (2.5 μg l^{-1}). Partially purified oyster odor was tested at levels that evoke creeping responses in snails at the same percentage as 2.5 μg l^{-1} barnacle odor. Cyprids were loaded into the upstream end of the tube which was then connected to a Gilmont #2 flow meter. A seawater flow of 6.8ml/min^{-1} was initiated (velocity gradient 39.4 sec^{-1}; Crisp, 1955) and continued for 2 minutes with collection of effluent. The flow was terminated after two minutes. The tube was disconnected and drained by the force of gravity (velocity gradient 606 sec^{-1}). Cyprids either investigated the surface of the tube (adhered), or they passed through the tube (did not adhere). Treatments were analyzed by G-test for goodness of fit (Sokal and Rohlf, 1969) comparing the number of cyprids adhering and the number not adhering.

LARVAL SETTLEMENT ASSAY

In settlement assays cyprids were portioned into glass or polystyrene containers with: 4 ml seawater with 10 μg/ml settlement factor, or 4 ml seawater containing barnacle or oyster odor. Partially purified oyster and barnacle odor were tested at concentrations equivalent to 25 μg protein l^{-1} to 0.8 μg protein l^{-1}. After 20 hours cyprids attached permanently (settled) and not attached permanently were counted and compared by contingency analysis. The same surface was used for all treatments in any particular experiment because the responses of larvae are surface dependent (Rittschof *et al.*, 1984c).

RESULTS

Cyprid behavior assays were used to compare settlement factor to purified barnacle or oyster odor and to examine responses of larvae to water from field environments with differing densities of barnacles. Cyprids behaved similarly in tubes coated with the chemically isolated settlement factor and with chemical from barnacle odor ($G=1.68$, ns). Treatments resulted in over 60% of the cyprids adhering to the tubes, as compared to 11% in the control. Differences between control and both treatments were highly ($p < .01$) significant (Table 1), demonstrating that chemicals, partially purified from barnacle odor were as effective as barnacle settlement factor when tested at similar protein concentrations. However, these experiments tested chemicals from odors at concentrations comparable to those of settlement factor. The concentration of odor used is 1000 fold higher than that known to exist in nature.

Table 1. Effect of settlement factor and snail attractant on barnacle cyprid behavior.

Treatment	Number Adhering	Number Not Adhering	G Value	Significance Level
Control	99	771	—	—
Settlement factor	373	283	363	< .001
Barnacle odor	356	231	395	< .001

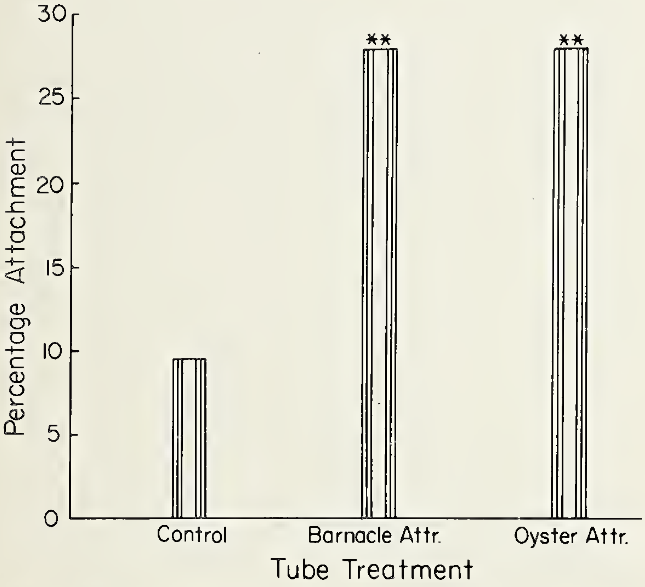

Fig. 1. Responses of larval barnacles to tubes onto which odors of barnacles (Barn Attr) and oysters (Oyst Attr) were adsorbed. The attractant labels indicate what I believe to be the major function of the odor cue. Adsorption was by 5 s exposure to solutions that make preparatory snails creep. Control was a five second exposure of tubes to seawater passed through a 100,000 Dalton filter. Duplicate assays were run and varied less than 3%. Frequency analysis of numbers of cyprids attaching in the various conditions shows significant ($p < 0.01$) attachment to tubes with adsorbed odors.

In a second experiment chemicals purified from barnacle odor and chemicals purified from oyster odor were tested in the behavior assay at concentrations known to evoke creeping in predatory snails, that is $2.5\,\mu g$ protein l^{-1}. This more accurately reflects the naturally occurring concentration of barnacle odor (Rittschof *et al.*, 1984b). At this concentration both barnacle odor and the corresponding dilution of oyster odor also promoted attachment of barnacle larvae (Fig. 1). These data suggest that concentrations of barnacle and oyster odor in the field are sufficient to elicit sticking behavior in larval barnacles.

In the next series of experiments assay tubes were rinsed in different field environments and tested in the behavior assay. Tubes were rinsed with seawater (control); water over a barnacle free sand flat; water from *Spartina alterniflora* containing a low density of barnacles; water from the immediate vicinity of a piling that was covered with a continuous sheet of barnacles and with sea water in which 5 g barnacles 100 ml^{-1} had incubated for 1 h. There was a trend toward increase in sticking behavior with increased amounts of barnacles. Significant increases ($G > 20$ 1 d.f. $p < 0.01$) in sticking behavior (over control levels) were observed in the tests with water from the barnacle-coated piling and from water in which barnacles had incubated (Table 2).

Larval barnacles were used in settlement assays to determine if purified prey odors from barnacle and oysters would induce metamorphosis. Settlement factor

Table 2. Responses of barnacle larvae to glass tubes rinsed in water from areas containing different densities of barnacles.

Treatment	Barnacle Density	% Stick	G Value	Significance Level
Control	0	6	—	—
Sand Flat	very low	7	< 1	NS
Spartina	low	10	< 4	NS
Piling	high	17	> 20	< 0.01
Barnacle odor	very high	28	> 20	< 0.01

Table 3. Effect of settlement factor and snail attractant on settlement of barnacle cyprids.

Treatment	Number Settling	Number Not Settling	*Control vs* G Statistic	*Treatment* Probability
Control	43	79	—	—
Settlement factor	63	36	16.7	< .001
Snail attractant	59	35	16.2	< .001

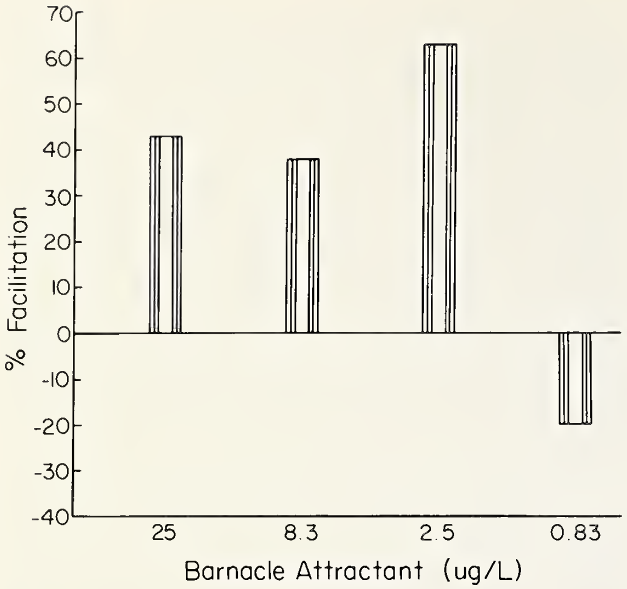

Fig. 2. Barnacle settlement in response to dilutions of barnacle odor peptides (barnacles attractant, see legend to Figure 1). Barnacle odor induced settlement at the three highest concentrations tested (analysis of settlement frequencies $p < 0.01$ overall $G > 35$ 4 d.f.). These concentrations are within the range of peptides present in natural waters where barnacles occur.

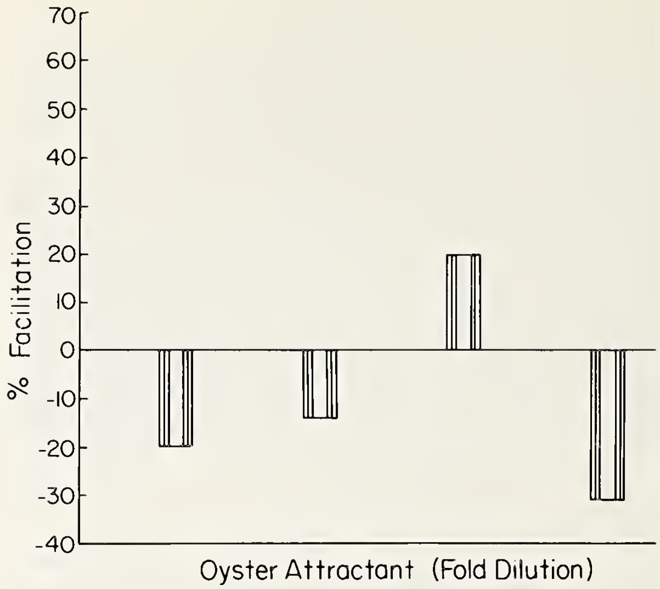

Fig. 3. Settlement of larval barnacles in the presence of odor from oysters (oyster attractant, see legend to Figure 1). Concentrations of oyster odor are comparable to barnacle odor concentrations (Fig. 2) as determined by responses of snails in the snail creeping bioassay. None of the concentrations of odor tested induced metamorphosis.

protein was used as an induction control because it is known to induce metamorphosis. A no addition seawater control was also included. This control indicated the frequency of settlement in noninduced larvae. There were no differences between settlement in the presence of 2.5μg protein ml^{-1} settlement factor and the presence of barnacle odor 2.5 μg protein ml^{-1} (G=0.001, ns). Settlement of treatment groups was over 60%, significantly greater than that observed for the no addition control (Table 3). In a second settlement assay dilutions of 25, 8, 2.5, and 0.8 μg protein l^{-1} of purified barnacle odor and the corresponding dilutions of oyster odor were tested. Cyprids used in these experiments were 1 day old. This age cyprid was used because it is maximally responsive to chemical stimulation (Rittschof *et al.*, 1984). Settlement was increased significantly from 28 to 67% above control levels in the presence of barnacle odor at the three highest concentrations. At the lowest concentration, 0.8μg l^{-1} (a level that evokes at best low level of creeping in the snail bioassay) settlement was equivalent to the no addition control (Fig. 2). Comparable dilutions of oyster odor did not result in chemical stimulation of metamorphosis (Fig. 3).

DISCUSSION

Isolation and purification of chemicals from prey odors that attract predatory snails has resulted in purification of a native form of barnacle settlement factor. As reported for gabamemetic peptides (Morse *et al.*, 1984), barnacle odors are extremely potent. These substances stimulate behavioral and physiological responses when present at the level of vertebrate hormones and insect pheromones. In addition to functioning as a cue for predators and a behavior signal for conspecific larvae, barnacle odor also acts at the physiological level to induce settlement. Evidence that cueing of surface investigation and induction of metamorphosis can be separated follows from two kinds of observations: 1) barnacle larvae respond behaviorally to oyster odor but oyster odor does not induce metamorphosis; 2) the capacity for chemical induction of metamorphosis is dependent upon larval age while investigatory behavior is independent of larval age (Rittschof *et al.*, 1984c). When larvae reach a certain physiological age barnacle and oyster odor cues should become equivalent. Thus, there appears to be a hierarchy of responsiveness based upon larval age. Optimal settlement is in the vicinity of successful conspecifics. However, at some point in time it becomes more adaptive to settle in the vicinity of other successful members of the fouling assemblage than to delay metamorphosis.

As demonstrated by the behavior assay (Table 1) barnacle cyprids detect settlement factor and prey odors adsorbed to surfaces (for review see Crisp, 1984). All evidence indicates that larval barnacles do not detect factors in solution (Crisp and Meadows, 1962; Crisp and Meadows, 1963; Rittschof *et al.*, 1984). Larval barnacles respond to prey odors adsorbed from solution concentrations measured in the field (Rittschof *et al.*, 1984b). There is a strong relationship between the density of odor generating animals and the strength of the behavioral response (Table 2). Thus, detection of odor on a surface results in investigation by larvae of surfaces in the proximity of living barnacles. Settlement factor adsorbed to surfaces also apparently improves the strength of permanent adhesion (Yule and Crisp, 1983).

Snails detect barnacle odors in solution (Carriker, 1955; Blake, 1962; Wood, 1968; Pratt, 1974; Brown and Rittschof, 1984). Detection in solution would increase the utility of the cue for a predator in distance location. Integration of flow and the soluble cue should indicate the present location of living barnacles. Thus, snails use a barnacle communication system to locate their barnacle prey.

Based upon our knowledge of snail and larval barnacle responses to prey odors, we predict that responses of larval barnacles to snail chemosuppressant substances (Williams *et al.*, 1983; Rittschof and Brown, 1985) and responses of larval bivalves to the entire suite of bioactive substances have intriguing potential for future study. It is already apparent that the complexity of chemical integration marine systems rivals that of insect dominated terrestrial systems.

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